Legacy and Replacement Flame Retardant Exposures Among Couples Seeking Fertility Treatment and Their Fertility Relevant Outcomes

by

Mary Elizabeth Ingle

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Doctoral Committee:

Professor John D. Meeker, Chair
Professor Russ Hauser, Harvard University
Assistant Professor Molly Moravek
Professor Marie O'Neill
Associate Professor Heather Stapleton, Duke University
Associate Professor Lu Wang

Mary Elizabeth Ingle

meingle@umich.edu

ORCID iD: 0000-0001-8392-2796

Dedication

I dedicate this dissertation to my daughter, Ina whose beautiful soul inspires me every day. Remember that it is our responsibility to do something in life to make the world more beautiful and that if we take care of the earth, it will take care of us.

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Abbreviations

ART	Assisted reproductive technologies
BCIPP	Bis(1-chloro-2-propyl) phosphate
BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate
BDCPP	Bis(1,3-dichloropropyl) phosphate
BDE	Brominate diphenyl ether
BDE47	2,2’,4,4’-tetrabromodiphenyl ether
BDE99	2,2’,4,4’,5-pentabromodiphenyl ether
BDE100	2,2’,4,4’,6-pentabromodiphenyl ether
BDE153	2,2’,4,4’,5,5’-hexabromodiphenyl ether
BDE154	2,2’,4,4’,5,6’-hexabromodiphenyl ether
CYPs	Cytochrome P450
DPHP	Diphenyl phosphate
E2	Estradiol
EARTH	Environment and Reproductive Health study
EU	European Union
FSH	Follicle stimulating hormone
HP	Household product
ICSI	Intracytoplasmic sperm injection
ip-PPP	Isopropylphenyl phenyl phosphate
IQR	Interquartile range
IVF	*In vitro* fertilization
LH	Luteinizing hormone
MGH	Massachusetts General Hospital
NBFR	Novel brominated flame retardants
NHANES	National Health and Nutrition Examination Survey
OH-BDE	Hydroxylated diphenyl ethers
OPE	Organophosphate ester
PBDE	Polybrominated diphenyl ethers
PCP	Personal care product
PGD	Preimplantation genetic diagnosis
SHBG	Sex hormone binding globulin
T3	Triiodothyronine
T4	Thyroxine
TBB	Bis(2-ethylhexyl-2,3,4,5 tetrabromobenzoate

TBPH	Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate
tb-PPP	Tert-butylphenyl phenyl phosphate
TCETP	Tris(2-chloroethyl) phosphate
TDCIPP	Tris(1,3-dichloroisopropyl) phosphate
TPHP	Triphenyl phosphate
TPP	Time to pregnancy
UK	United Kingdom
US	Unites States

Abstract

Infertility, the inability to conceive after one year of unprotected intercourse, affects approximately one out of every seven couples in the United States (US). In the last decade, 7.3 million Americans reported using fertility services. Infertility limits the likelihood of a live birth, but has also been associated with other reproductive diseases, psychosocial stress, and economic hardship. An infertility diagnosis can arise from female or male factors, or a combination of both. Currently, the use of assisted reproductive technologies (ART) including *in vitro* fertilization (IVF) is steadily on the rise.

Human reproduction is a composite of multiple biological responses which occur in phases, many of which occur without observation. Each phase is susceptible to exposures to various environmental toxicants, including flame retardants (FR) commonly found in furniture foams, carpeting, electronics, and plastics. Their lack of covalent bonds allows for leaching into the surrounding environment and thus lead to widespread exposure. Although some FRs have been phased out of production, they persist in the environment and the use of alternative compounds is increasing. Legacy FRs such as polybrominated diphenyl ethers (PBDEs) and replacement FRs like organophosphate esters (OPEs), have been widely detected among the US population. Laboratory studies suggest PBDEs and OPEs are reproductive toxicants, yet human data are lacking. Furthermore, studies suggest that some PBDE metabolites, such as

hydroxylated brominated diphenyl ethers (OH-BDEs), may elicit greater toxic effects compared to their respective parent compounds.

The three aims of this dissertation were executed using data from a subset of couples from the Environment and Reproductive Health (EARTH) study, an existing longitudinal pre-conception cohort assessing the impact of environmental, dietary and lifestyle factors on fertility among couples from a fertility clinic in Boston. The first aim evaluated the relationships of serum PBDE congeners and OH-BDE metabolites with early developmental (intermediate) and pregnancy (clinical) IVF outcomes among women seeking fertility treatment. The second aim investigated the joint effects of PBDE and OH-BDE exposure of women and their male partners with intermediate and clinical IVF outcomes. The final aim evaluated (1) whether exposure to OPEs as determined by levels of their urinary metabolites, was associated with in-home exposures and (2) the associations of OPE metabolites with male fertility.

We observed a steady decline in PBDE concentrations over the ten-year study period. Concentrations of all FRs were higher in women compared to their male partners. We observed unexpected positive associations between female PBDE and OH-BDE concentrations and clinical IVF outcomes. However, when also accounting for male exposure, effect estimates diminished and lost statistical significance. We observed a slight increase in OPE metabolite concentrations in the final years of recruitment. The use of several personal care and household products were associated with elevated OPE metabolite concentrations among couples. However, associations of OPE metabolites with male fertility were weak and inconsistent.

This dissertation highlights the persistence of PBDEs and their metabolites despite being phased out of production. Furthermore, our novel study design underscores the importance of considering male exposures when evaluating the relationships of environmental toxicants with fertility and pregnancy outcomes. This work highlights the persistence of FRs and suggests PCPs can be an important source of OPE exposure and warrants future investigations of their possible reproductive toxicity.

Chapter I

Introduction

Impact of Infertility

Infertility, the inability to conceive after one year of unprotected intercourse, affects one out of every seven couples in the United States (US), while 10% of couples experience some type of difficulty trying to conceive [1,2]. Between 2006-2010, a US national survey reported 12% of women are burdened by impaired fecundity, the biological capacity to become pregnant [3]. The underlying cause of infertility may be related to female or male factors or a combination of both. Known causes of female infertility include anatomical, hormonal, lifestyle, environmental, or unexplained factors [4]. Advanced maternal age has also been identified as a negative predictor of both fecundity and fertility, which is cause for concern as the number of women over the age of 30 having their first child has increased 20% in the last 30 years [5–8]. In 2002, approximately 3.2 million US men reported ever using fertility services [9]. However, a national survey study suggests this to be an underestimate for the US population as male factor infertility is likely to be underdiagnosed [9,10]. The cost of male factor infertility alone was $17 million US dollars in the year 2000, which does not include the additional $18 billion for assisted reproductive technology (ART) treatment [11]. To date, a semen analysis measuring sperm count, concentration, morphology, and volume remains the

primary evaluation for male factor infertility [10,12]. Semen quality is also associated with other various health outcomes including testicular cancer and decreased life expectancy [13,14]. A recent meta-analysis found an approximate 50% reduction in total sperm count and sperm concentration among men from Western countries over the last several decades, irrespective of infertility diagnosis [15]. Infertility not only prevents live birth, but has been associated with reproductive cancers, psychosocial stress, financial burden, and life expectancy [13,14,16–18].

Since 1981, ART has aided those suffering from infertility [19]. A large proportion of ART is through *in vitro* fertilization (IVF), a process of *in vitro* oocyte fertilization and embryo culture, followed by embryo transfer through the cervix into the uterus [2]. In 2013, approximately 7.3 million people reported ever using infertility services [3]. These numbers are likely to rise as the number of ART cycles (excluding those initially intended for cryopreservation) in the US increased 13% from 2013 to 2015 [19,20]. Advanced maternal age, genetic risk factors, other health issues, and psychosocial factors can impair fertility and increase the use of ART services [7,21]. Exposure to environmental toxicants has also been associated with infertility [22]. In the European Union (EU), health care costs associated with infertility as a result of environmental exposures alone was estimated at €1.8 billion euros [23].

Flame retardant exposure in the general population

Polybrominated diphenyl ethers (PBDEs)

Over the last 75 years, flame retardants (FRs) with ranging chemical compositions have been added to thousands of consumer products to protect people, property, and the environment [24]. FRs operate by several mechanisms including reducing ignition and suppressing the spread of fire. Approximately 2.5 billion pounds of FRs are either manufactured or imported into the US annually [25]. Additive FRs lack a covalent bond to their original materials which allows them to leach into the environment [26]. The most prominent class of FRs added to consumer products from the 1970s to mid-2000s were polybrominated diphenyl ethers (PBDEs) due to their high efficiency and cost effectiveness [27]. PBDEs were added to carpeting, electronics, and plastics and constitute up to 30% of product by weight [26,28,29]. There are 209 specific PBDE congeners with various bromine substitution patterns, yet the most frequently detected congeners in the environment and humans are from three commercial mixtures referred to as, Penta-, Octa-, and Deca- brominated diphenyl ether (BDE). The distribution of specific congeners by weight for the three commercial mixtures is presented in Figure l.1. However, due to toxicity in humans and ecosystems, the US voluntarily phased out the use of PentaBDE and OctaBDE mixtures in 2004 while DecaBDE was not phased out until 2013 [30].

Pharmacokinetic models suggest the daily body burden of PBDEs in the early 2000s to be 7.7ng/kg body weight and 33.8 ng/kg lipid weight [31]. PBDEs are lipophilic with half-lives ranging from weeks to years depending on the specific congener and bioaccumulate in the environment, which has led to ubiquitous exposure [32]. Typically, PBDEs with a lower degree of bromination are more resilient to biological degradation and continue to persist in the environment [33,34]. The primary route/pathway of exposure

in the US is via dust ingestion from indoor environments, although exposure through diets high in poultry and red meat is most prominent in the EU [30,31,35]. Congeners from the PentaBDE mixture have been frequently detected (67-100%) in dust samples from homes and offices in Boston, MA [36,37]. Similarly, congeners 47, 99, 100, 153, and 154 are the most frequently detected in humans [30]. However, PBDE concentrations have been decreasing over time. The National Health and Nutrition Examination Survey (NHANES) observed decreases for congeners 47, 99, and 100 from 2005-2014 [38].

Hydroxylated polybrominated diphenyl ethers (OH-BDEs)

In mammals, hydroxylated-BDEs (OH-BDEs) are formed through oxidative metabolism of PBDEs via cytochrome P450 (CYPs) [39]. However, OH-BDEs are also biogenic and produced in marine environments and contribute to internal exposure through seafood consumption [40]. Similar to their parent compounds, OH-BDEs are lipophilic and have been detected in the serum of adults. The most frequently detected metabolites in humans are 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, and 4-OH-BDE49 [39,41–43]. Limited human studies to date have assessed the toxicity of OH-BDEs. However, several laboratory studies suggest OH-BDEs act as endocrine disruptors and elicit greater toxic responses compared to PBDEs [44–47]. One study found OH-BDEs to elicit a significantly greater thyroid hormone responses compared to PBDEs [48]. Another study observed OH-BDEs inhibit transthyretin and estradiol-sulfotransferase 160-1600 and 2.2-200 times higher compared to BDE47, one of several parent compounds [49]. A study of pubertal boys observed no comparable differences in concentrations between parent compound and metabolite whereas a study of Japanese women found

concentrations of 6-OH-BDE47 to be 20-fold higher than one of its parent compounds, BDE47 [41,50].

Organophosphate esters (OPEs)

Since the phase-out of PBDEs in the mid-2000s, the prevalence of organophosphate esters (OPEs) has risen drastically and they are increasingly being used as replacement FRs in furniture foams, baby products, cars, electronics, and carpeting [51–54]. However, some non-chlorinated alkyl and aryl phosphates are used as plasticizers and antifoaming agents [55–57]. Triphenyl phosphate (TPHP), a non-halogenated aryl phosphate, and chlorinated alkyl esters such as tris(2-chloroethyl) phosphate (TCEP), and tris(1,3-dichloroisopropyl) phosphate (TDCIPP) are commonly used to comply with flammability standards and frequently added to lacquers, paints, glues, and hydraulic fluids [58,59]. As the use of PBDEs diminished, the preference for OPEs increased and in 2008, they became a high production volume chemical; US production is projected to reach 50,000 tons in 2020 [60].

During manufacturing, OPEs are physically mixed with other compounds and not covalently bound to the product(s). The lack of a covalent bond allows these semi-volatile compounds to escape into the environment via leaching, abrasion, or volatilization [58,61,62]. Exposure to OPEs is dependent upon their respective physicochemical properties and specific application. OPEs have been detected in the dust of homes, cars, and offices and primary exposure is likely through dust ingestion [31,35,36]. Recent studies suggest possible routes/pathways through inhalation and dermal absorption also contribute to exposure [63–66]. Despite half-lives ranging from

hours to days, metabolites have been detected in nearly 100% of urine samples of children and adults [58,62,67,68]. Frequently detected OPE metabolites include bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), and tert-butylphenyl phenyl phosphate (tb-PPP) [67,68]. Exposure to OPEs has been associated with oxidative stress, and disruption of endocrine, metabolic, immune, and reproductive systems [66,69,70]. TCEP and TDCIPP are also known carcinogens [71].

Flame retardants as a reproductive toxicant

Several laboratory studies have also examined the effects of PBDEs on reproduction, primarily through endocrine disruption [72,73]. Male rats exposed during gestation to low doses of BDE99 (60 or 300 µg) had reduced sperm and spermatid counts, while female offspring experienced a mixture of up and down regulation of estrogen receptors [74,75]. An analysis in a previous sample of men (n=24) from the Environment and Reproductive Health (EARTH) cohort found a 30% decrease in follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in association with congeners 47, 99 and 100 measured in house dust [76]. However, all congeners were positively associated with an increase in inhibin B (30%) and sex hormone binding globulin (SHBG) (18%). A larger follow up study observed an increase in SHBG, estradiol (E_2), free thyroxine (T4) and total (triiodothyronine) T3, yet decreasing FSH with an interquartile range (IQR) increase in congeners 47, 99, and 100 [36]. BDE47 concentrations in serum was also associated with increased testosterone levels among sport fisherman (n=405) of the Great Lakes [77].

Few human studies have investigated the relationship of PBDEs and female reproductive health. However, longer menstrual cycles (>32 days) were associated with increased levels of BDE153 in breast milk [78]. Detectable concentrations of serum BDE153 was also associated with an increase in the odds of failed implantation among a sample of women (n=65) seeking IVF treatment in Boston, MA [79]. In a study of women in California, congeners 47, 99, 100, 153, and their sum measured in serum were all associated with a longer time to pregnancy (TTP) [26]. Associations of PBDE exposure with semen quality are mixed. While some report associations of decreased motility and morphology, others have observed no associations [80–82]. However, the inconclusive findings could potentially be a result of the cross-sectional study design. Studies investigating the association of combined PBDE exposure of couples (male and female) exposure and fertility outcomes are lacking.

To date, studies assessing the reproductive health effects of OPEs are limited, yet animal and *in vitro* studies suggest these compounds act as endocrine disrupting chemicals. A study of TPHP and TCEP in mice observed a disruption of gene expression for testosterone synthesis and oxidative stress, while an *in vitro* study of mouse Leydig cells observed a disruption in steroid production [83,84]. A small study of US men detected inverse relationships of urinary metabolite concentrations of BDCPP and DPHP with sperm concentration and motility [85]. Paternal urinary concentrations of BDCIPP was associated with a decrease in fertilization among couples seeking fertility treatment [86]. However, among a sample of women seeking fertility treatment, the sum of several OPEs (BDCIPP, DPHP, ip-PPP, and tb-PPP) measured in urine was associated with declines in implantation, clinical pregnancy, and live birth [69].

Despite the high prevalence of exposure and laboratory studies suggesting PBDEs are a reproductive toxicant, human fertility studies are lacking. Most research to date has focused on the disruption of reproductive hormones. Only one study (n=65) has studied PBDEs among the IVF population, and was solely focused on embryo implantation [79]. Similarly, few studies have assessed the role of PBDEs in males during IVF. To date, no studies have assessed the reproductive toxicity of PBDEs in comparison to their hydroxylated metabolites. However, *in vitro* models have shown OH-BDEs to be estrogen agonist and genotoxic [46,87,88].

Studies assessing the health effects of OPEs are limited, yet laboratory studies also suggest these compounds act as endocrine disrupting chemicals. While the majority of human studies have focused on exposure of children and pregnant women, only one small study has evaluated OPE exposure with male fertility outcomes [85]. While OPEs have been detected in dust, no study has assessed their relationship with household products (HP), and studies with personal care products (PCPs) are limited. One study identified TPHP in eight out of ten nail polish samples and observed a 7-fold increase in DPHP (metabolite of TPHP) after polish application (n=26) [56].

Specific AIMS

Human reproduction is multifaceted, and each stage is susceptible to various environmental toxicants to which humans are exposed, including FRs. The aim of this dissertation is to build upon the limited existing knowledge of the reproductive toxicity of FRs and to identify the specific biological endpoints these insults may be associated with among couples seeking fertility treatment. A conceptual diagram outlining the

specific aims of this dissertation is illustrated in Figure I.2. The first two aims of this dissertation will build upon existing research of PBDE exposure and couples undergoing IVF by (1) focusing on more intermediate endpoints (i.e. response to hormone interventions, number of oocytes retrieved, and embryo quality) of IVF among females and (2) assessing PBDE and OH-BDE exposure among 'couples' rather than females and males separately. The third aim of this dissertation will (1) expand upon the few studies assessing the reproductive toxicity of OPEs in humans and (2) determine if certain PCPs and HPs are predictive of OPE exposure using a repeated measures study design.

Specific Aim #1. To investigate the relationship between maternal serum concentrations of PBDEs and OH-BDEs with IVF outcomes. More specifically, to evaluate the association of congeners: 47, 99, 100, 154, and 153 and OH-BDEs: 4-OH-BDE49, 6-OH-BDE47, 5-OH-BDE47, 3-OH-BDE47 with intermediate IVF outcomes using generalized linear mixed models (GLMM) with random effects and cluster weighted generalized estimating equations (CWGEE) models.

Hypothesis #1: Increased PBDE concentrations in maternal serum will be negatively associated with IVF outcomes.

Hypothesis #2: OH-BDEs will have stronger associations IVF outcomes compared to PBDEs.

Specific Aim #2: To investigate the joint relationship between PBDE and OH-BDE concentrations in both maternal and paternal serum with IVF outcomes. Specifically, to

evaluate the association of paternal PBDE and OH-BDE concentrations in serum with fertilization rate and clinical IVF endpoints while also adjusting for female serum concentrations and covariates using GLMM and CWGEE.

Hypothesis #3: The joint effects (compared to each individually) of serum PBDEs in 'couples' serum will have stronger associations with negative clinical outcomes compared to individual exposure.

Hypothesis #4: The joint effects of OH-BDEs in 'couples' will have stronger associations with negative clinical outcomes compared to PBDEs.

Specific Aim # 3: To investigate the relationship between men and women seeking fertility treatments and exposure to five urinary OPE metabolites: BCIPP, BDCIPP, DPHP, ip-PPP, and tb-PPP. Product use will be identified from patient questionnaires as predictors of OPE metabolite concentrations in urine. Male fertility parameters (sperm count, concentration, motility, and morphology) were used to assess the relationship with OPE metabolites.

Hypothesis #5: Reported use of certain household and personal care products will be predictive of elevated exposure concentrations of urinary OPE metabolites in females and males.

Hypothesis #6: Increased exposure to urinary PFR metabolites will be negatively associated with clinical measures of male fertility.

Study Population

This dissertation is comprised of a subset of study participants from the EARTH study, an established longitudinal prospective pre-conception cohort of couples from a fertility center where we study the impacts of environmental, dietary, and lifestyle factors on reproductive health. Women (n=230) and their respective male partners (n=229) were recruited at the Massachusetts General Hospital (MGH) fertility center in Boston, MA between 2005-2016. Selected fertility center characteristics are described in Tables I.1 and I.2. Among all ART cycles at MGH in 2013, 100% utilized IVF (n=803 cycles). Approximately 50% of those cycles utilized intracytoplasmic sperm injection (ICSI) or preimplantation genetic diagnosis (PGD) [89]. Participants were recruited at their initial visit to MGH fertility center regardless of treatment. Questionnaires were administered to collect demographic information (i.e. age, race/ethnicity, education level) at study entry. Both men (18-55 yrs.) and women (18-46 yrs.) were eligible to participate. However, men who had previously undergone a vasectomy were ineligible. Participants are followed for a maximum of 6 IVF cycles (mean= 1.4).

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Table I. 1. Selected Massachusetts General Hospital (MGH) fertility center pregnancy success rates from fresh embryos from patient's oocytes, 2013

IVF cycles (n=803)	
Procedure	%
ICSI	45
Unstimulated	0
PGD	4
Diagnosis	%
Female factor	38
Male factor	18
Female & male factor	20
Other factor	4
Unknown	19

ICSI: Intracytoplasmic sperm injection; PGD: Preimplantation genetic diagnosis; Female factors include: tubal, ovulatory dysfunction, diminished ovarian reserve, endometriosis, uterine factor, and multiple female factor; Results modified from the 2013 Clinic Summary Report for MGH [90]. Note: these are characteristics of all MGH fertility clinic patients, not only those in the EARTH study.

Table I. 2. Selected age-stratified outcomes from fresh embryos (non-donor eggs) from MGH Fertility Center, 2013.

	Maternal age categories				
	<35	35-37	38-40	41-42	>42
Number of cycles	215	140	148	83	18
Cycles resulting in pregnancies (%)	57.7	46.4	36.5	32.5	16.7
Cycles resulting in live birth (%)	53.0	40.7	29.7	20.5	11.1
Cycles with elective single embryo transfer (%)	34.9	19.4	3.0	1.4	0
Implantation rate (%)	49.2	31.3	20.5	10.6	5
Average number of embryos transferred	1.6	1.8	2.4	3.6	4

Results modified from the 2013 Clinic Summary Report for MGH [90]. Note: these are characteristics of all MGH fertility clinic patients, not only those in the EARTH study. Throughout recruitment (2005-2016), MGH clinic shifted to single embryo transfer.

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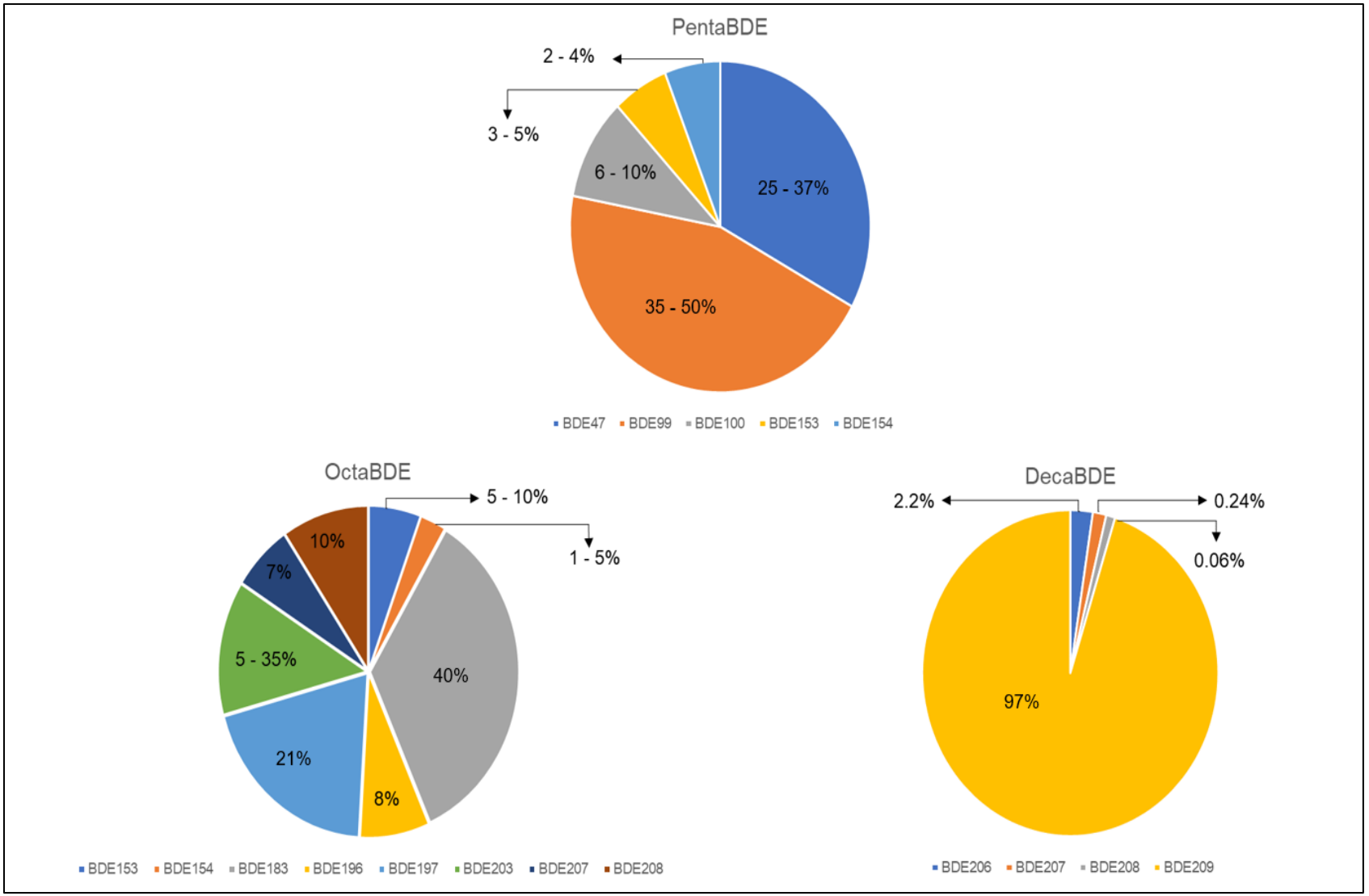
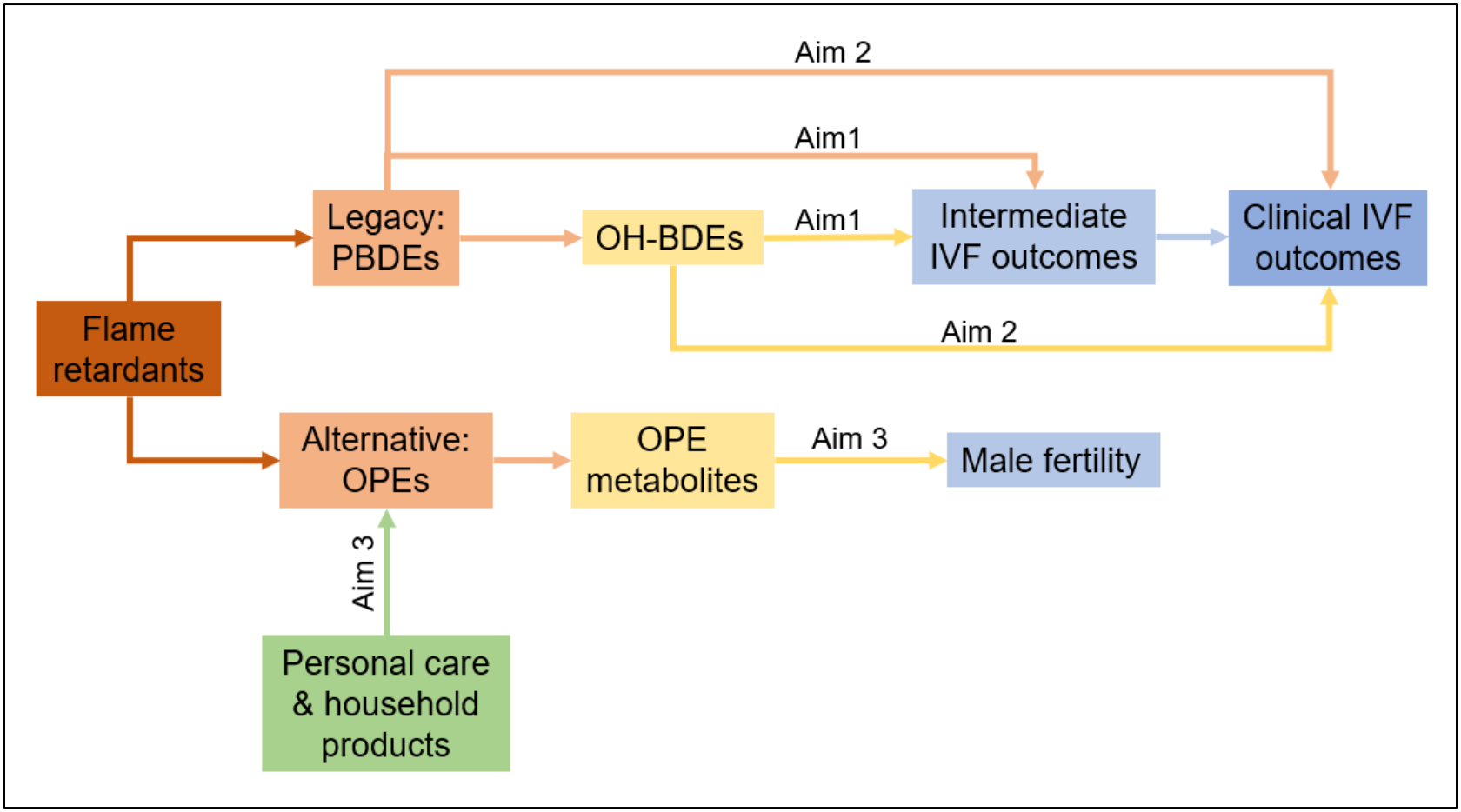
Figure I.1. Distribution of specific congener composition for commercial brominated diphenyl ether mixtures.



Adapted from the ASTDR's Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs), 2015

Figure. I.2. Conceptual diagram of specific aims.



Intermediate IVF outcomes: Total oocyte yield, M2 oocyte yield, endometrial wall thickness, and fertilization rate; Clinical IVF outcomes: Implantation, clinical pregnancy, and live birth; Male fertility characterized by sperm: count, concentration, motility, progressive motility, and morphology

CHAPTER II

AIM 1: Exploring Reproductive Associations of Serum Polybrominated Diphenyl Ether and Hydroxylated Brominated Diphenyl Ether Concentrations Among Women Undergoing *In Vitro* Fertilization

Abstract

Background: Polybrominated diphenyl ethers (PBDEs) have been voluntarily phased out of production in the US and EU due to their persistence and toxicity to humans and ecosystems. PBDEs have been associated with implantation failure among women undergoing IVF, yet some animal studies suggest greater toxicity from their metabolites, hydroxylated brominated diphenyl ethers (OH-BDEs). The objective of this study is to investigate the associations of serum concentrations of polybrominated diphenyl ethers PBDEs and OH-BDEs with *in vitro* fertilization endpoints (IVF).

Methods: We evaluated a subset of 215 women (contributing 330 IVF cycles) enrolled between 2005-2016 in a longitudinal cohort based at Massachusetts General Hospital Fertility Center. The following PBDEs were quantified: 47, 99, 100, 153, and 154 and the following OH-BDEs: 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, and 4-OH-BDE49. Clinical endpoints of IVF treatments were abstracted from electronic medical records. Associations of log-transformed PBDEs and OH-BDEs with IVF outcomes were assessed using multivariable generalized mixed models and cluster weighted

generalized estimating equation models adjusted for lipids, age, BMI, race, year of sample collection, IVF protocol, and follicle stimulating hormone levels.

Results: Detection frequencies were highest for congeners 47 and 153 (82% ≥ method detection limit (MDL)) and metabolites 3 and 5-OH-BDE47 and 4-OH-BDE49 (92%>MDL). PBDE and OH-BDE geometric mean concentrations declined by up to 80% over the study period. An interquartile range (IQR) increase in BDE153 was associated with an increase in the probability of implantation (Relative risk (RR) =1.26, 95% CI: 1.16, 1.36), clinical pregnancy (RR= 1.32, 95% CI: 1.19, 1.46), and live birth (RR= 1.34; 95% CI: 1.15, 1.54). An IQR increase in 3 and 5-OH-BDE47 was associated with increased probabilities of implantation (RR= 1.52; 95% CI:1.11, 2.09), clinical pregnancy (RR= 1.66; 95% CI: 1.17, 2.36), and live birth (RR= 1.61; 95% CI:1.07, 2.40). When models were stratified by race (White (86%)/Other race (14%)), associations remained positive for White women, yet inverse associations were observed for Other race women. An IQR increase in BDE47 was associated with a 46% decreased probability of clinical pregnancy (95% CI: 0.31, 0.95) for Other race women.

Conclusion: Detections of serum concentrations of PBDEs and OH-BDEs were highest in the early years of the study and suggests the phase-out of these compounds has contributed to a decrease in exposure. The negative associations found for PBDEs and IVF outcomes among other race women suggests the potential for racial disparity. Potential racial disparities in PBDE exposure and exploration of alternative flame retardants with reproductive health outcomes should be the focus of future investigations.

Introduction

Approximately 15% of couples in the US and 80 million couples worldwide are affected by infertility, defined as the inability to conceive after one year of unprotected intercourse [1–3]. The annual number of treatment cycles using assisted reproductive technology (ART) increased by 13% from 2013-2015. In 2016, 1.8% of all live births in the US were a result of ART. Infertility treatment (including diagnosis and sequelae) in the US is suspected to cost $5 billion annually, which is easy to conceptualize as one cycle of *in vitro* fertilization (IVF) has an average cost of $12,400. Environmental exposures including particulate matter, heavy metals, pesticides, and persistent organic pollutants (POPs) have been associated with infertility [4–6].

Among POPs that have been associated with infertility are polybrominated diphenyl ethers (PBDEs), a prominent class of flame retardants (FR) found in furniture, carpeting, electronics, and plastics [7]. While there are potentially 209 different brominated diphenyl ether (BDE) congeners (having various bromine substitution patterns), only a handful of congeners are routinely detected in the environment and in human tissues, and reflect the dominant congeners present in the commercial mixtures referred to as Penta-, Octa-, and Deca- BDE. The most frequently detected congeners, 47, 99, 100, 153, and 154 are often found in (but not exclusively) the PentaBDE mixture [8]. OctaBDE is primarily comprised of congeners 183, 196, 197, and 203 (153 and 154 can be found in PentaBDE and OctaBDE), while 207 and 208 can be found in both OctaBDE and DecaBDE, but 202 and 209 are exclusively found in DecaBDE [7]. Due to these mixtures' toxicity to humans and ecosystems, PentaBDE and OctaBDE mixtures were phased out of production in 2004 and DecaBDE mixtures were phased out in 2013

[9]. During manufacturing, PBDEs are physically added to the polymers and the absence of a covalent bond allows them to leach into surrounding environments [10]. Their lipophilicity allows them to bioaccumulate in the environment and adipose tissues with half-lives ranging from weeks to years depending on the specific congener, which has led to widespread detection in serum among women, men, and children in the US [11]. PBDEs have been highly detected in dust samples from homes, offices, and automobiles and it is suspected that approximately 82% of exposure in the US can be attributed to house dust, while exposure in Europe is primarily from food [12–14].

In mammals, hydroxylated-BDEs (OH-BDEs) are formed through oxidative metabolism of PBDEs via cytochrome P450 (CYPs), specifically CYP2B6 [15]. OH-BDEs are also produced naturally in marine environments and exposure can also be attributed to seafood consumption [16]. Like PBDEs, OH-BDEs also accumulate in the body and have been detected in human serum in adults and children [17–19]. Several laboratory studies have also observed greater toxic effects from OH-BDEs compared to their parent compounds in regards to endocrine disruption, cytotoxicity, and genotoxicity [20–24]. The most common metabolites detected in humans are 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, and 4-OH-BDE49 [15,17–19].

Elevated levels of PBDEs have been associated with adverse reproductive health effects in humans, including endocrine disruption, longer menstrual cycles, and TTP [18,25–28]. [27]. To date, studies assessing the reproductive health effects of OH-BDEs are limited; however, several *in vitro* studies suggest they act as endocrine disruptors and elicit oxidative stress [20,22,23,29]. In our present work we expand upon previous studies in a more robust analysis investigating the association of PBDEs and pregnancy

outcomes along with, to the best of our knowledge, the first study to assess these relationships with OH-BDEs using IVF as a model of intermediate developmental endpoints and pregnancy outcomes.

Methods

Study Population

Study participants are a subset of women from the EARTH study, an established longitudinal prospective pre-conception cohort study of environmental, dietary, and lifestyle impacts on reproductive health [30]. Women (18-46 years) were recruited from Massachusetts General Hospital (MGH) Fertility Center. Approximately 60% of women who were approached enrolled in the study [31]. Questionnaires were administered to collect demographic information (i.e. age, race/ethnicity, education level) at study entry. The present analysis includes 215 women (330 IVF cycles) recruited between 2005-2016 who contributed their own oocytes and provided a blood sample to quantify concentrations of FRs and FR metabolites in serum. The majority of women completed 1-2 IVF cycles during follow up. Research protocols were approved by the ethics and Research Committees of MGH, Harvard T.H. Chan School of Public Health, University of Michigan, and Duke University. The study was described in detail to all participants and informed consent was obtained from all participants.

Clinical protocols and IVF measures

During each cycle, clinical data was abstracted from the participant's electronic health record. Clinical protocols and IVF measures have been previously detailed [31,32]. Briefly, on the third day of the woman's menstrual cycle, a blood serum sample was

drawn to measure follicle stimulating hormone (FSH) and estradiol (E_2) concentrations at MGH Core Laboratory using an automated electrochemiluminescence immunoassay. Peak E_2 concentrations, defined as the highest level prior to oocyte retrieval, were obtained on the day of trigger with human chorionic gonadotropin (hCG). Infertility diagnosis was established by an MGH physician in accordance with the Society of Assisted Reproductive Technology (SART) [33]. Upon infertility evaluation including infertility diagnosis and other clinical factors, one of three ovarian treatment IVF protocols was selected: (1) luteal phase gonadotrophin releasing hormone (GnRH) agonist, (2) follicular phase GnRH agonist or "flare" stimulation, or (3) GnRH antagonist [34]. Throughout gonadotropin stimulation and up to two days prior to oocyte retrieval, serum E_2, follicle size and counts, and endometrial thickness were monitored for each participant [31]. Once lead follicle size approached 16-18 mm and E_2 levels reached at least 500 pg/mL, oocytes were retrieved. IVF cycle was defined by oocyte retrieval. Following oocyte retrieval, an embryologist counted and classified oocytes per cycle as a germinal vesicle, metaphase I, metaphase II (M2), or degenerated. Fertilization occurred via IVF or intracytoplasmic sperm injection (ICSI), and was confirmed 17-20 hours later. Fertilization was determined by the presence of a cytoplasmic halo and two pronuclei [35,36]. Fertilization rate was established as the number of two pronuclear embryos divided by the number of M2 oocytes. Clinical outcomes were assessed for participants who continued with embryo transfer. Successful implantation for a given transfer was confirmed when serum β-hCG levels were > 6 mIU/mL, approximately 17 days after oocyte retrieval [31]. Clinical pregnancy was defined as the presence of an

intrauterine pregnancy via ultrasound (≥ 6 wks. gestation) along with elevated levels of β-hCG. Live birth was defined as the birth of a neonate at or after 24 weeks gestation.

PBDE and OH-BDE collection and measurement

Blood samples (5 mL) were collected in washed glass Wheaton vials at the study entry clinic visit. Samples were aliquoted, frozen and stored at -80° C until overnight shipment on dry ice to Dr. Stapleton's lab at Duke University (Durham, NC). Serum was analyzed for five PBDE congeners: 47, 99, 100, 153, and 154 along with four OH-BDE metabolites: 4-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, and 4-OH-BDE49. Upon arrival, samples were weighed and fortified with internal standards (monofluorinated BDE 69, 13C BDE 209, and 13C-6-OH-BDE47). Serum was diluted with water and formic acid before undergoing sold phase extraction (Oasis HLB, Waters Corp.). A 50:50 solution of dichloromethane (DCM) and ethyl acetate was used to remove both PBDES and OH-BDEs from the SPE column. Samples were then dried and rejuvenated with hexane (1 mL) followed by extract cleaning via a 1.0 g silica column. PBDEs were removed with 10 mL of hexane while 10 mL of DCM hexane solution was used for the metabolites. Gas chromatography negative chemical ionization mass spectrometry methods were used to analyze PBDES while OH-BDEs were measured using liquid chromatography tandem mass spectrometry [18]. Accuracy of the method was verified by extracting a human serum Standard Reference Material (SRM 1957) from the National Institute of Standards and Technology. Measured values were in the range of 73%-97% of the certified values. Metabolites 3-OH-BDE47 and 5-OH-BDE47 are presented individually as well as combined due to co-elution problems in a few batches. Total lipids were derived from total serum cholesterol and triglycerides using the following

formula: TL (g/l) = [(TC x 1.12) + (TG x 1.33) + 1.48] where TL= total lipids, TG= serum triglycerides, and TC= serum cholesterol [37]. Missing total lipids (n=19) were replaced with the median (511.5).

Statistical analysis

Demographic and reproductive characteristics for women were calculated using medians, interquartile ranges (IQRs), frequencies, and percentages as appropriate. Congeners and metabolites below method detection limits (MDL) were imputed to MDL/$\sqrt{2}$ [38]. Geometric means (GM), 95% confidence intervals (CIs), and selected percentiles were used to describe unadjusted (ng/g serum) and lipid-adjusted (ng/g lipid) PBDEs and OH-BDEs. Spearman correlation coefficients were used to assess the association among serum congeners and metabolites. PBDEs and OH-BDEs presented as right skewed and were transformed by the natural logarithm for further analysis. Associations of congeners and metabolites with reproductive outcomes were confirmed to meet linearity assumptions using bivariate analyses and were treated as continuous variables. Possible demographic confounders included total serum lipids, age, body mass index (kg/m^2) (BMI), race (White/Other race), education (high school/some college, college graduate, graduate degree), and year of exposure sample collection. Reproductive characteristics considered were prior pregnancy, day 3 FSH levels (IU/L), initial infertility diagnosis (female factor, male factor, or unexplained), previous intrauterine insemination (IUI) (yes/no), previous IVF (yes/no), treatment protocol (antagonist, flare, or luteal phase agonist), E_2 trigger levels (pmol/L), endometrial thickness (mm), and ICSI (yes/no). Covariates were selected if they were associated with exposure in previous cohorts, our cohort, or a predictor of IVF outcomes

[18,28,39,40]. Final models using unadjusted PBDEs and OH-BDEs were adjusted for total serum lipid, age, BMI, race, year of exposure sample collection, day 3 FSH levels, and IVF protocol. A missing value (n=1) for day 3 FSH level was replaced with the median (6.8 IU/L).

We evaluated the associations of PBDEs and OH-BDES with intermediate IVF outcomes (total oocyte yield, M2 oocyte yield, endometrial wall thickness, and fertilization rate) using multivariable generalized mixed models with random intercepts. A Poisson distribution with log link function were designated for oocyte counts while a normal distribution with identity link function was designated for endometrial thickness. A binomial distribution and logit function were designated for fertilization rate. Associations with PBDEs and OH-BDEs with clinical outcomes (implantation, clinical pregnancy, and live birth) were evaluated using cluster weighted generalized estimating equation (CWGEE) models where the weight was equivalent to the inverse of the cluster size (cycle number) [41,42]. CWGEEs have some advantages when accounting for multiple cycles per women (i.e. non-ignorable cluster size), since they may provide more precise estimates when compared to other common statistical approaches for dichotomous IVF outcomes [43]. PBDEs and OH-BDEs were modeled individually as well as summed. Metabolites 3-OH-BDE47 and 5-OH-BDE47 were initially modeled individually, however due to co-eluting for some batches we decided to present their findings as a collective sum (3-OH-BDE47, 5-OHBDE47, 3-OH-BDE47 & 5-OH-BDE47). Original effect estimates are presented in tables, and to increase interpretability, outcomes in figures and text were adjusted to represent a percent change in outcome

with an IQR increase in PBDE or OH-BDE which was calculated using the formula: $((75^{th}$ percentile/25^{th} percentile$)^{\beta} - 1) \times 100$ [44].

We conducted several sensitivity analyses. To further explore our observation of higher PBDE and OH-BDE concentrations among women in the first half of the study, we stratified our analysis by year (<2010, ≥2010) and reran our models. We also reran our models stratified by race (White or Other) as prior studies have observed variability in PBDE exposure by race [18]. Analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and R version 3.3.5.

Results

Demographic and reproductive characteristics of this cohort (n=215 women) are described in Table 1 and are similar to previous subsets from the EARTH cohort [31,35]. Our sample was comprised of predominantly White women (86%) with a normal BMI (median=23) in their mid-thirties (median=35 years). Few women (28%) reported ever smoking and over half had graduate degrees (61%). Only 36% of women reported having a prior pregnancy. The majority of initial infertility diagnoses were male factor (36%), followed by unexplained (35%), then female factor (29%). More women had undergone a previous IUI (39%) compared to IVF (21%). Median day three FSH levels were 6.8 IU/L (IQR: 6.0-8.3). Considerably more women underwent luteal phase agonist protocol (69%), compared to flare (18%), and antagonist (13%). The median peak E_2 levels were 1998 pmol/L and endometrial thickness was 10 mm. Approximately half of cycles (52%) underwent ICSI.

Distributions of PBDEs and OH-BDEs are presented in Table 2 as unadjusted (ng/g serum) and lipid-adjusted (ng/g lipid). Congeners 47, 100, and 153 were frequently detected (70%>MDL). Concentration levels (GM) of BDE47 and BDE153 were approximately five times greater than BDEs 99 and 100. Metabolites 3-OH-BDE47, co-eluted samples of 3-OH-BDE47 and 5-OH-BDE47, and 4-OH-BDE49 were frequently detected (92%>MDL). The combined concentrations of 3-OH-BDE47 and 5-OH-BDE47 were the highest (GM= 1.3 ng/g lipid) of all metabolites. 3-OH-BDE47 concentrations (GM= 0.32 ng/g lipid) were slightly higher compared to 6-OH-BDE47 and 4-OH-BDE49 (GM= 0.19 ng/g lipid and GM= 0.20 ng/g lipid).

Yearly trends of lipid-adjusted PBDE and OH-BDE concentrations (GMs) throughout the study are shown in Figure II.1. Concentrations were highest for BDE47 (GM=67 ng/g/ lipid) and BDE154 (GM=20 ng/g lipid) in 2005. BDE99 peaked in 2008 (GM= 9 ng/g lipid), while BDE100 and BDE153 did not peak until 2009 (GM=8 ng/g lipid and GM=54 ng/g lipid). By 2014, concentrations of congeners 99, 100, and 153 had decreased by 66, 63, and 63% (GM=3 ng/g lipid, GM=3 ng/g lipid, and GM=20 ng/g lipid, respectively). Between 2005-2015 concentrations of BDE154 decreased by 50%, while concentrations of BDE47 decreased by almost 80%. Similar trends were observed for OH-BDES. 3-OH-BDE47 and 5-OH-BDE47 concentrations decreased by 80% over the study period, yet had a slight peak in 2010 (GM=37 ng/g lipid). Although initial concentrations of 6-OH-BDE47 and 4-OH-BDE49 were not as high at the beginning of the study compared to other metabolites, concentrations still decreased substantially (75% and 57%, respectively).

Several demographic and clinical characteristics were associated with PBDE and OH-BDE exposure (data not shown). Correlations between BMI and BDE100 were positive (r=0.15), yet negative for BDE153 (r=-0.15). Other race women had higher concentrations of BDE47, BDE99, 3 and 5-OH-BDE47, and 4-OH-BDE49 compared to White women. Current or past smokers had higher concentrations of BDE47 and BDE99 than non-smokers. Nulliparous women had higher concentrations of congeners 99, 100, and 153.

Correlations for lipid-adjusted PBDEs and OH-BDEs (ng/g lipid) are presented in Figure II.2. All PBDEs were significantly correlated with each other. Correlations were strongest for BDE47, BDE99, and BDE100 (0.73-0.85), while correlations for BDE153 and BDE154 were slightly weaker (0.31-0.53). Moderate-to-strong correlations (0.39-0.56) were observed for all OH-BDEs. Metabolites had the strongest correlations to BDE47 (3 and 5-OH-BDE47: r=0.57, 6-OH-BDE47: r=0.47, and 4-OH-BDE49: r=0.64). Strong correlations were also observed with 4-OH-BDE49 and BDE99 (r=0.59) and BDE100 (r=0.47). Associations were weakest for BDE154 and 4-OH-BDE49 (r=0.30).

No associations were observed for any PBDEs with intermediate IVF outcomes (prior to implantation) in unadjusted and adjusted models (Tables II.A.1 and II.A.2). However, in adjusted models, an IQR increase of 4-OH-BDE49 was associated with a 39 and 48% increase (95%CI: 0.5, 94 and 95% CI: 5, 109%, respectively) in total oocyte and M2 oocyte yield (Table II.A. 4).

Associations of PBDEs and OH-BDEs with clinical IVF outcomes are depicted in Figures 3 and 4 and represent the percent change in outcome in relation to an IQR increase in PBDE/OH-BDE concentrations. For example, an IQR increase in BDE153

was associated with increased probabilities of implantation, clinical pregnancy, and live birth (RR=1.16; 95% CI: 1.16, 1.36, RR= 1.25; 95% CI: 1.16, 1.35, RR= 1.26; 95% CI: 1.13, 1.41). An IQR increase in 3 and 5-OH-BDE47 was associated with increased probabilities of implantation (RR= 1.28; 95% CI:1.07, 1.53), clinical pregnancy (RR= 1.35; 95% CI: 1.11, 1.64), and live birth (RR= 1.31; 95% CI:1.04, 1.66). An IQR increase in 6-OH-BDE47 was also associated with a 56% increase in the probabilities of both implantation and clinical pregnancy, and an 84% increase in the probability of live birth (RR= 1.56, 95% CI:1.14, 2.14, RR= 1.56; 95% CI: 1.12, 2.18, and RR= 1.84, 95% CI: 1.26, 2.68, respectively).

When stratifying by race (White/Other race), associations with BDE153 remained positive for clinical outcomes among White women (Figure II.A.1). An IQR increase in BDE47 was associated with a decreased probability of implantation (36%), clinical pregnancy (46%), and live birth (35%) for Other race (RR= 0.64; 95% CI: 0.36, 1.16, RR= 0.54; 95% CI: 0.31, 0.95, and RR=0.65; 95% CI: 0.38, 1.13, respectively), while associations for White participants remained positive. We also observed a decrease in the probability of clinical outcomes with congeners 99, 100, and 153 for Other race women. We also observed several non-significant changes in models of OH-BDEs with clinical outcomes when stratifying by race (Figure II.A.2).

Discussion

Serum concentrations of PBDEs among women in this cohort decreased over the span of the study; they were highest between 2005-2009 and decreased substantially from 2010-2015. There were no consistent associations for PBDEs and metabolites with intermediate IVF outcomes. We observed unexpected positive relationships of

BDE153, 3 and 5-OH-BDE47, and 6-OH-BDE47 with clinical IVF outcomes. Positive associations remained for White women in the relationships of BDE153, and 3 and 5-OH-BDE47 with clinical outcomes. However, among Other race women, an increase in congeners 47, 99, 100, 154 and metabolite 4-OH-BDE49 was associated with a decreased probability of clinical IVF outcomes. Although the study included a very small number of Other race women, these results suggest a possible racial disparity in PBDE exposure outcome relationships.

Serum concentrations of PBDEs in our cohort were higher compared to the general population. From a similar time period (2005-2014), concentrations in our cohort of BDE47 (GM=28.1 ng/g lipid), BDE153 (GM=26.3 ng/g lipid), and BDE154 (GM=7.3 ng/g lipid) were higher than those from pooled samples of women (20-59 years) from the National Health and Nutrition Examination Survey (NHANES) (GM=21.0 ng/g lipid, GM=7.9 ng/g lipid, and GM= 0.4 ng/g lipid, respectively) [45]. Concentrations of BDE47 among our sample were also nearly double those among pregnant women in California (CA) (GM=14.9 ng/g lipid) and North Carolina (NC) (GM=16.5 ng/g lipid) [18,28]. Of the congeners in our sample, BDE47 is the most abundant in the PentaBDE mixture (25-37%) and possibly explains the higher concentrations of BDE47 compared to other congeners in our sample [7]. Concentrations of BDE99 (GM=5.5 ng/g lipid) and BDE100 (GM=5.1 ng/g lipid) in our sample were similar to women in CA (GM=4.4 ng/g lipid and GM=2.8 ng/g lipid, respectively) and NC (GM=4.7 ng/g lipid and GM=4.2 ng/g lipid). Concentrations of BDE47 among participants of this study (median=24.6 ng/g lipid) were nearly five-fold higher compared to women in a different cohort of women seeking fertility treatment in Boston between 1994-2003 (median=5.22 ng/g lipid) and nearly

three-fold higher among reproductive aged women in Canada (GM=9.0 ng/g lipid) between 2007-2009 [39,46]. BDE153 concentrations in our sample were also considerably higher compared to women in Canada (1.4 ng/g lipid). Higher PBDE concentrations in our cohort could be due to different sampling time periods, as recent serum concentrations may be lower due to the phase out compared to samples taken in the early 2000s. Differences in BDE153 levels may also be attributable to changes in diet over time and across geographic region.

Few studies to date have analyzed PBDE metabolites. Pregnant women in NC had slightly lower concentrations of 6-OH-BDE47 (GM=0.11 ng/g lipid) compared to participants of this study (GM=0.19 ng/g lipid) [18]. Concentrations of 4-OH-BDE49 among our sample were similar (GM=0.20 ng/g lipid) to women in NC (GM=0.17 ng/g lipid). However, among pregnant women in Indiana, concentrations were higher for 5-OH-BDE47 (median=5.7 ng/g lipid) and 6-OH-BDE47 (GM=1.0 ng/g lipid) compared to participants of this study (median<MDL and median=0.18 ng/g lipid, respectively). Yet 3-OH-BDE47 (median=0.31 ng/g lipid) and 4-OH-BDE49 (median=0.21 ng/g lipid) were higher in our sample compared to women in IN (median=0.4 ng/g lipid and not detected, respectively).

Concentrations decreased by year of sample collection. This trend correlates to the phase-out of the PentaBDE mixture from US markets in 2004 as BDEs 47, 99, 100, and 154 were highest in 2005 [47]. A similar trend was also observed for a recent decade- long (2005-2014) analysis of pooled PBDE samples from NHANES where concentrations for congeners 47, 99, 100, and 154 were lower in recent years for people between the ages of 20-59 years [48]. A longitudinal study of pregnant women in

CA also observed a steady decrease in congeners 47, 99, 100, and 153 between 2008-2014 [49].

Correlations were moderate-to-strong (r=0.30-0.85) among PBDEs, OH-BDEs, and between PBDEs and OH-BDEs. Congeners 47, 99, and 100 had the strongest correlations, while congeners 153 and 154 were slightly weaker. These results were expected as congeners 47, 99, and 100 are the most prevalent congeners (by weight) in the PentaBDE mixture followed by BDEs 153 and 154 [7]. Similar results were observed in serum among a sample of 137 pregnant women in NC, where BDE47 was strongly correlated to BDE99 (r=0.80) and BDE100 (r=0.80), yet weaker for BDE153 (r=0.52) [18]. However, among a serum sample of men and women in Shanghai, China (n=25), BDE47 was strongly correlated to both BDE100 (r=0.97) and BDE154 (r=0.97) [50]. They also observed a strong correlation between BDE100 and BDE154 (r=0.99). Correlations for OH-BDEs were slightly weaker among our sample ($0.39 \leq r \leq 0.59$). Correlations between 6-OH-BDE47 and 4-OH-BDE49 among pregnant women in NC were slightly higher (r=0.62). All metabolites in this analysis are found in the hydroxylation pathway of BDE47, which coincides with our observation of the strongest correlations between BDE47 and OH-BDEs ($0.47 \leq r \leq 0.64$) [19]. Similar correlations were observed in serum among a sample (n=47) of women in Dalian, China ($0.26 \leq r^2 \leq 0.65$) [51].

Our results for associations of PBDES and OH-BDEs with intermediate IVF outcomes were overall null while results with clinical outcomes were positive, though unexpected. An increase in BDE153, 3 and 5-OH-BDE47, and 6-OH-BDE47 was associated with an increased probability of implantation, clinical pregnancy, and live

birth. These results are unexpected as prior studies of IVF patients found an increase in failed implantation (Odds ratio (OR) =10.0) with detectable BDE153 concentrations in follicular fluid (FF) [39]. Another study found increases in BDE100 and BDE153 to be associated with a decrease in fecundability odds ratio (fOR) (fOR=0.6 and fOR=0.5, respectively) [28]. In all clinical models, metabolites had stronger and positive associations with clinical outcomes compared with the parent compound, BDE47. These results were unanticipated as previous studies suggest OH-BDEs to be more toxic than PBDEs due to their disruption of oxidative phosphorylation, which is associated with fertilization and early embryo development [52–54].

Although concentrations decreased for PBDEs and OH-BDEs over the study period, we did find a slight increase in BDE 47 and 153 along with 3 and 5-OH-BDE47 and 6-OH-BDE47 concentrations between 2007-2010. Coinciding with this time period, we also observed an increase in the likelihood of successful implantation, clinical pregnancy, and live birth rates in our cohort (Figure II.A.4). Increasing IVF success rates over time could have possibly biased our results of PBDEs/OH-BDEs with clinical outcomes. Our results could also be due to residual confounding. Although we adjusted for year of sample collection in our original models and performed a stratified analysis based on year (data not shown), it is possible these quantifications of year did not appropriately account for the associations for women who underwent multiple cycles within the same year compared to their PBDE and OH-BDE concentrations measured at study entry. Seafood consumption possibly mitigated our results with OH-BDEs and IVF endpoints as omega-3 polyunsaturated fatty acids (i.e. seafood consumption) have been associated with positive IVF outcomes, although results are mixed [55]. A study of

Japanese women found a 20-fold increase in concentrations of 6-OH-BDE47 compared to BDE47, likely attributed to seafood consumption [56].

When clinical outcomes were stratified by race, on average among Other race women the probabilities of implantation, clinical pregnancy, and live birth all decreased with increased concentrations of all PBDEs and OH-BDES, except for 6-OH-BDE47. However, only the association between BDE47 and a decreased probability of clinical pregnancy reached statistical significance, which could be due to the small number of Other race women (and cycles) in our sample. Several studies have established racial and ethnic disparities in exposure to endocrine disrupting chemicals, including PBDEs among women [57]. We observed higher concentrations of PBDEs among Other race women (Figure II.A.3). Similar results were observed from NHANES, which found higher levels of BDE47 and BDE99 among Mexican Americans and Blacks compared to Whites [11]. A larger analysis of NHANES data observed higher concentrations of BDE47 and BDE99 among non-Hispanic Blacks compared to all other race and ethnicity groups [48]. Higher concentrations of six PBDE congeners have been observed among US Black adolescent girls compared to Whites [58].

Our study is not without limitations. Despite the long half-lives of PBDEs ranging in the order of years, it is possible for exposure misclassification for women who underwent multiple cycles (over months or even years) with only a single exposure measurement [59]. Like many other studies, we measured PBDE and OH-BDEs in serum, while the measurement of chemicals in FF could potentially be an optimal medium for the specific microenvironments for reproductive studies [60,61]. A previous IVF study comparing PBDEs in FF and serum detected weak but significant correlations

(Kendall's Tau-beta (T_b) =0.15-0.38) for congeners 47, 100 and 154 [39]. We also tested associations for multiple congeners and metabolites with multiple outcomes. Lastly, a limitation of the study is that the results were very imprecise for the stratified analyses. This could be due to the small sample size among women of Other race (n=30), but confidence intervals were also wide for models conducted within White women. The homogeneity of our population, similar to other IVF cohorts, may have resulted in a lack of precision of the probabilities of clinical outcomes seen in the Other race stratified models [62]. However, we also observed wider confidence intervals for White models.

To the best of our knowledge, the present study is the largest prospective preconception cohort assessing the association of PBDEs and OH-BDEs on reproductive health. This is also the first study to assess the relationship between PBDEs and OH-BDEs with intermediate IVF outcomes (prior to implantation). An IVF cohort also allows for the study of many endpoints critical to a successful pregnancy but not observable in a TTP study in the general population. Our prospective study design eliminates the possibility of reverse causation. Finally, our use of a CWGEE model for clinical outcomes account for the multiple cycles per woman and provide more precise effect estimates compared to other statistical approaches [43].

Conclusion

Among our cohort, PBDEs and OH-BDEs were frequently detected in serum with concentrations highest in the early years of the study period, which coincides with the phase out of the PentaBDE mixture in 2004 [47]. We did not observe any consistent trends of PBDEs or OH-BDEs with intermediate IVF outcomes but identified some unexpected positive relationships of clinical IVF outcomes with BDE153, 3 and 5-OH-

BDE, and 6-OH-BDE. Our stratified models supported prior studies of racial disparities with higher concentration of PBDEs among Other race populations. Future studies should focus on the rise in use of alternative FRs as concentrations of PBDEs continue to drop as well as employ designs to adequately explore racial or ethnic disparities with PBDE exposure and their associations with reproductive health.

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Table II.1. Demographic and reproductive characteristics for 215 women (330 *in vitro* fertilization cycles) from a subset of the EARTH cohort.

Characteristics	Median or n	(IQR or %)
Demographic (n=215 women)		
Age (years)	35	(32, 38)
Race/ ethnicity		
Other race	30	(14)
White	185	(86)
Body mass index (kg/m^2)	23	(21, 26)
Ever smoker	60	(28)
Education		
High school/some college	14	(6)
College graduate	70	(33)
Graduate degree	131	(61)
Reproductive (n=330 cycles)		
Prior pregnancy	78	(36)
Initial infertility diagnosis		
Female factor	63	(29)
Male factor	77	(36)
Unexplained	75	(35)
Previous IUI	83	(39)
Previous IVF	46	(21)
Day 3 FSH levels [a], IU/L	6.8	(6.0, 8.3)
Treatment protocol		
Antagonist	42	(13)
Flare	60	(18)
Luteal phase agonist	228	(69)
E_2 trigger levels [b], pmol/L	1998	(1540, 2658)
Endometrial thickness [c] (mm)	10	(8.5, 11.2)
ICSI cycles	164	(52)

[a] n=329; [b] n=317; [c] n=316; IQR: Interquartile range; Other race: Black/Asian/Other BMI: Body mass index; IUI: intrauterine insemination; FSH: Follicle stimulating hormone; E_2: Estradiol; ICSI: Intracytoplasmic sperm injection

Table II.2. Distribution of unadjusted and lipid-adjusted BDEs and OH-BDEs among 215 women from the EARTH cohort.

					Percentiles			
BDEs	N>MDL	(%)	GM	(95% CI)	25th	50th	75th	95th	Max
Unadjusted (pg/g serum)									
BDE47	175	82.5	12.5	(11.1, 14.2)	6.3	11.1	24.1	70.7	191.2
BDE99	122	59.2	2.5	(2.2, 2.8)	<MDL	2.0	4.5	12.3	67.4
BDE100	149	71.0	2.3	(2.1, 2.6)	<MDL	1.9	3.9	14.3	42.8
BDE153	201	94.0	12.0	(10.5, 13.8)	6.2	10.3	22.7	80.1	256.5
BDE154	108	53.1	3.1	(2.8, 3.4)	<MDL	3.3	4.8	10.7	26.7
BDE sum			38.7	(34.9, 42.9)	21.4	34.8	68.0	160.9	287.0
Lipid-adjusted (ng/g lipid)									
BDE47			28.1	(25.3, 31.3)	13.4	24.6	57.8	145.9	326.5
BDE99			5.5	(4.9, 6.1)	2.5	5.0	10.6	31.4	162.7
BDE100			5.1	(4.6, 5.7)	2.3	4.2	10.8	29.9	73.0
BDE153			26.3	(23.3, 29.7)	13.0	24.5	47.7	172.3	690.3
BDE154			7.3	(6.7, 7.9)	4.8	7.0	12.2	24.7	62.3
BDE sum			86.3	(78.6, 94.7)	44.2	78.9	157.8	365.4	758.7
OH-BDEs									
Unadjusted (pg/g serum)									
3-OH-BDE47 [a]	76	93.8	0.14	(0.11, 0.19)	0.07	0.12	0.36	0.92	2.0
3-OH-BDE47 & 5-OH-BDE47 [b]	132	98.5	0.59	(0.49, 0.72)	0.29	0.60	1.3	3.3	9.9
5-OH-BDE47 [a]	1	1.0	0.01	(0.01, 0.01)	<MDL	<MDL	<MDL	<MDL	0.81
6-OH-BDE47	134	62.3	0.09	(0.07, 0.10)	<MDL	0.08	0.21	0.96	2.3
4-OH-BDE49	198	92.0	0.09	(0.08, 0.11)	0.04	0.09	0.18	0.62	1.7
OH-BDE sum			0.65	(0.56, 0.74)	0.30	0.62	1.3	4.0	10.7
Lipid-adjusted (ng/g lipid)									
3-OH-BDE47 [a]			0.32	(0.27, 0.41)	0.13	0.31	0.94	1.8	3.7
3-OH-BDE47 & 5-OH-BDE47 [b]			1.3	(1.1, 1.5)	0.64	1.4	2.6	8.1	19.1
5-OH-BDE47 [a]			0.02	(0.01, 0.02)	<MDL	<MDL	<MDL	<MDL	1.7
6-OH-BDE47			0.19	(0.17, 0.22)	0.08	0.18	0.45	1.6	4.2
4-OH-BDE49			0.20	(0.18, 0.23)	0.09	0.21	0.44	1.2	4.1
OH-BDE sum			0.33	(0.29, 0.37)	0.16	0.35	0.74	2.1	5.6

MDL: Method detection limit; GM: Geometric mean; CI: Confidence interval; [a] n=81; [b] n=134

Figure II.1. Geometric means of BDEs and OH-BDEs (ng/g lipid) from 215 women from the EARTH cohort by year (2005-2015) of sample collection. (Only one sample was collected in 2016 and not included.)

Number of samples per year: 2005: n=4, 2006: n=15, 2007: n=16, 2008: n=35, 2009: n=47, 2010: n=46, 2011: n=48, 2012: n=39, 2013: n=36, 2014: n=32, 2015: n=8

Figure II.2. Spearman correlation coefficients of BDEs and OH-BDEs (ng/g lipid) among 215 women from the EARTH cohort.

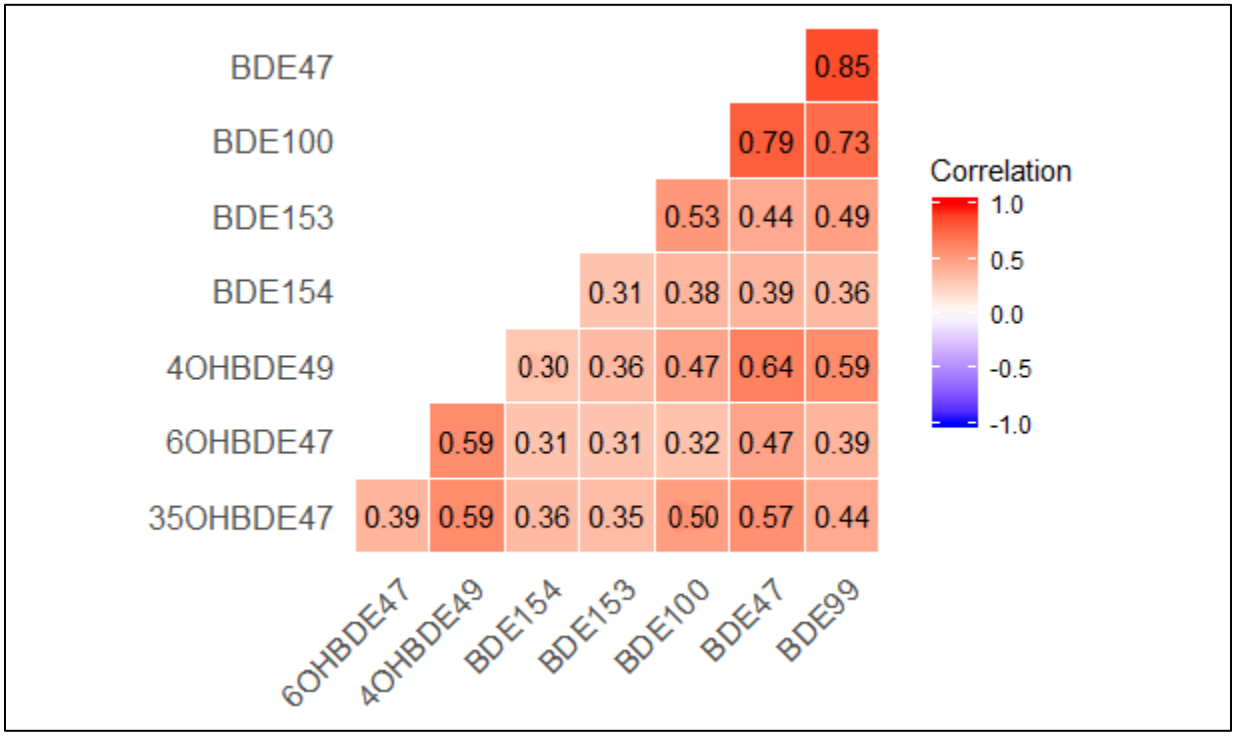
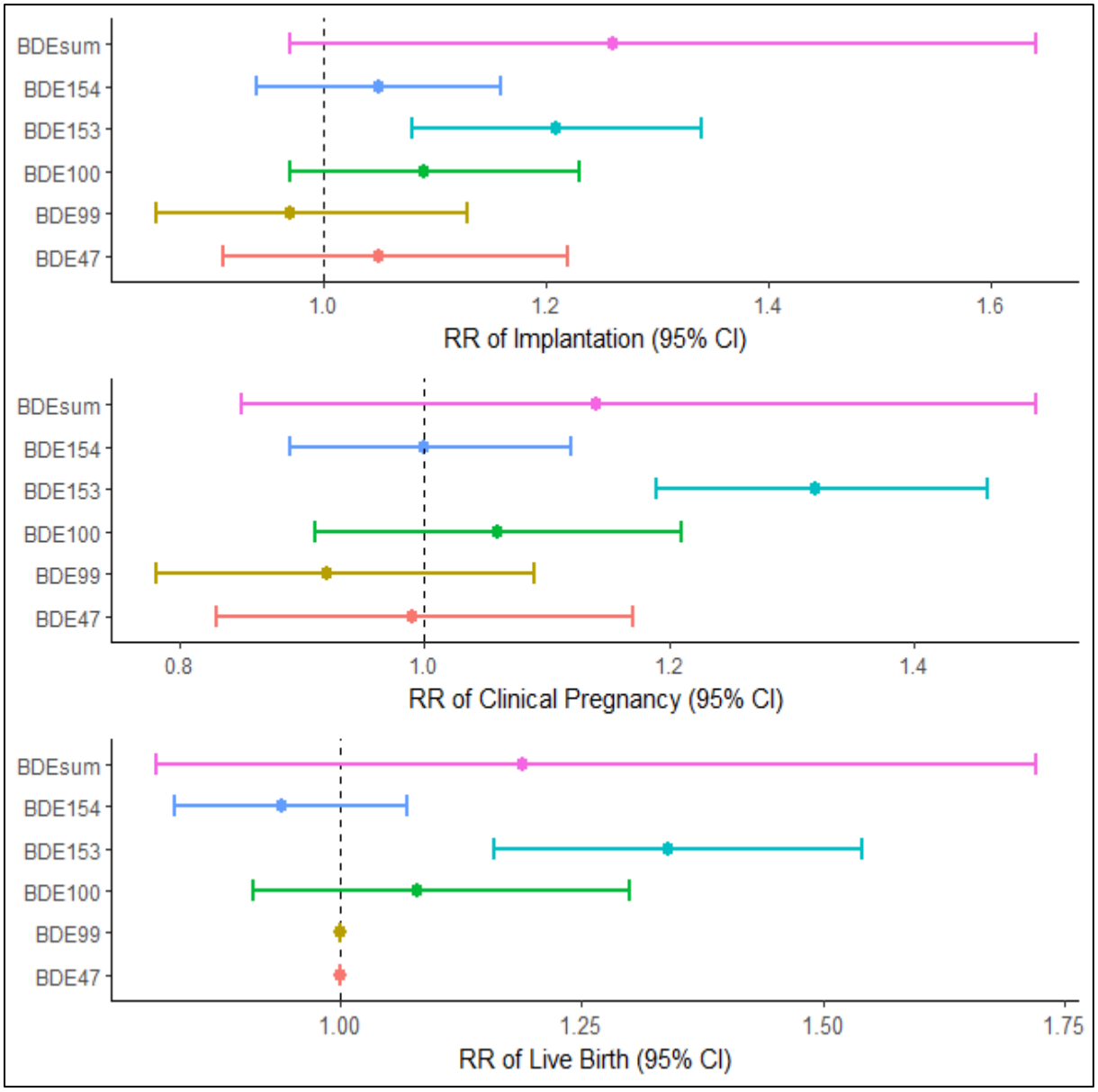
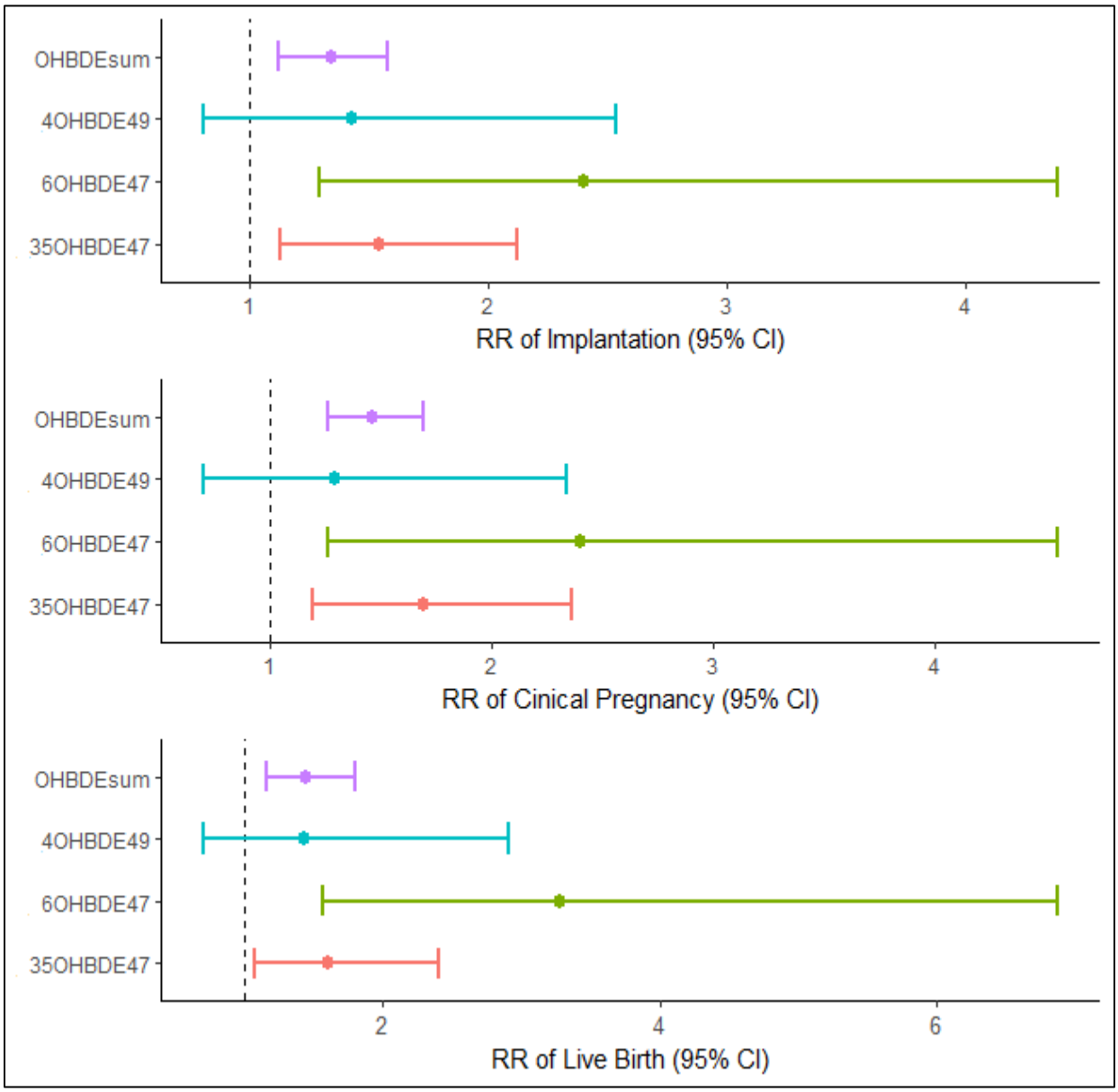


Figure II.3. Adjusted relative risk (RR) (95% CIs) for clinical outcomes among women with an interquartile range increase in BDE concentrations (ng/g serum).



Models adjusted for total serum lipid, age, BMI, race (White/Other race), year of BDE sample collection, day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist). RR: Relative risk.

Figure II.4. Adjusted relative risk (RR) (95% CIs) for clinical outcomes among women with an interquartile range increase in OH-BDE metabolites concentrations (ng/g serum).



Models adjusted for total serum lipid, age, BMI, race (White/Other race), year of BDE/OH-BDE sample collection, day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist). RR: Relative risk.

Chapter II Appendix

Table II.A.1. Unadjusted regression coefficients and relative risks (95%) CI for the association of PBDEs (ng/g serum) and IVF outcomes from a subset of 215 women (330 IVF cycles) from the EARTH cohort.

	PBDEs [a]											
	BDE47		BDE99		BDE100		BDE153		BDE154		BDE Sum	
Intermediate outcome [b]	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)
Total oocyte yield [c]	-0.02	(-0.09, 0.05)	-0.03	(-0.01, 0.04)	-0.02	(-0.09, 0.05)	-0.01	(-0.07, 0.04)	-0.08	(-0.17, 0.005)	-0.08	(-0.22, 0.05)
M2 oocyte yield [c]	0.01	(-0.06, 0.08)	-0.002	(-0.07, 0.07)	-0.01	(-0.08, 0.06)	-0.01	(-0.07, 0.05)	-0.08	(-0.17, 0.01)	-0.04	(-0.17, 0.10)
Endometrial wall thickness [c]	-0.16	(-0.49, 0.17)	-0.20	(-0.54, 0.14)	-0.25	(-0.57, 0.08)	0.08	(-0.21, 0.37)	0.13	(-0.29, 0.55)	-0.15	(-0.80, 0.50)
Fertilization rate [d]	0.02	(-0.12, 0.16)	-0.03	(-0.17, 0.12)	0.11	(-0.03, 0.24)	0.04	(-0.08, 0.16)	0.03	(-0.14, 0.21)	0.13	(-0.14, 0.40)
Clinical outcome [e]	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
Implantation	1.05	(0.81, 1.38)	0.97	(0.75, 1.26)	1.12	(0.87, 1.45)	1.35	(1.07, 1.72)	1.08	(0.77, 1.53)	1.36	(0.81, 2.30)
Clinical Pregnancy	0.96	(0.75, 1.24)	0.92	(0.71, 1.18)	1.08	(0.84, 1.38)	1.36	(1.09, 1.72)	0.96	(0.69, 1.34)	1.15	(0.70, 1.91)
Live birth	1.08	(0.83, 1.40)	1.02	(0.79, 1.32)	1.16	(0.90, 1.49)	1.42	(1.12, 1.79)	0.80	(0.57, 1.13)	1.22	(0.72, 2.06)

[a] Natural log transformation; [b] Generalized linear mixed models; [c] n=316; [d] n=314; [e] Cluster weighted generalized estimating equations; M2: Metaphase 2; RR: Risk ratio

Table II.A.2. Regression coefficients and relative risks (95%) CI for the association of PBDEs (ng/g serum) and IVF outcomes from a subset of 215 women (330 IVF cycles) from the EARTH cohort.

	PBDEs [a]											
	BDE47		BDE99		BDE100		BDE153		BDE154		BDE Sum	
Intermediate outcome [b]	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)
Total oocyte yield [c]	-0.01	(-0.08, 0.06)	-0.03	(-0.10, 0.04)	-0.03	(-0.10, 0.04)	-0.02	(-0.08, 0.04)	-0.04	(-0.13, 0.05)	-0.07	(-0.21, 0.06)
M2 oocyte yield [c]	0.01	(-0.06, 0.08)	-0.005	(-0.08, 0.07)	-0.02	(-0.09, 0.05)	-0.02	(-0.08, 0.04)	-0.05	(-0.14, 0.03)	-0.04	(-0.18, 0.10)
Endometrial wall thickness [c]	-0.18	(-0.54, 0.18)	-0.20	(-0.56, 0.16)	-0.30	(-0.64, 0.05)	0.13	(-0.18, 0.43)	0.25	(-0.20, 0.70)	-0.11	(-0.82, 0.60)
Fertilization rate [d]	0.04	(-0.11, 0.19)	-0.01	(-0.16, 0.14)	0.13	(-0.02, 0.27)	0.04	(-0.09, 0.16)	0.05	(-0.14, 0.23)	0.15	(-0.13, 0.44)
Clinical outcome [e]	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
Implantation	1.04	(0.93, 1.17)	0.98	(0.87, 1.10)	1.07	(0.97, 1.20)	1.16	(1.06, 1.26)	1.07	(0.91, 1.26)	1.22	(0.97, 1.54)
Clinical Pregnancy	0.99	(0.87, 1.13)	0.94	(0.82, 1.07)	1.04	(0.92, 1.19)	1.25	(1.16, 1.35)	1.00	(0.84, 1.19)	1.11	(0.87, 1.43)
Live birth	1.00	(1.00, 1.00)	1.00	(1.00, 1.00)	1.08	(0.92, 1.26)	1.26	(1.13, 1.41)	0.92	(0.76, 1.11)	1.16	(0.83, 1.61)

[a] Natural log transformation; [b] Generalized linear mixed models adjusted for total serum lipid, age, BMI, race (White/Other race), year of BDE sample collection, Day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist); [c] n=316; [d] n=314; [e] Cluster weighted generalized estimating equations adjusted for total serum lipid, age, BMI, race (White/Other), year of BDE sample collection, Day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist); M2: Metaphase 2; RR: Risk ratio

Table II.A.3. Unadjusted regression coefficients and 95% CI for the association of OH-BDEs and IVF outcomes from a subset of 215 women (330 IVF cycles) from the EARTH cohort.

	OH-BDEs [a]							
	3-OH47 & 5-OH47		6-OH47		4-OH49		OH-BDE Sum	
Intermediate outcome [b]	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)
Total oocyte yield [c]	0.08	(-0.09, 0.25)	-0.04	(-0.52, 0.43)	-0.01	(-0.58, 0.56)	0.04	(-0.08, 0.15)
M2 oocyte yield [c]	0.01	(-0.08, 0.11)	-0.01	(-0.22, 0.21)	0.28	(0.04, 0.50)	0.03	(-0.04, 0.10)
Endometrial wall thickness [c]	0.21	(-0.25, 0.67)	0.85	(-0.19, 1.89)	0.71	(-0.40, 1.83)	0.26	(-0.07, 0.60)
Fertilization rate [d]	-0.14	(-0.33, 0.05)	0.19	(-0.24, 0.62)	-0.04	(-0.51, 0.43)	-0.06	(-0.19, 0.08)
Clinical outcome [e]	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
Implantation	1.71	(1.16, 2.51)	3.07	(1.27, 7.42)	1.92	(0.75, 4.91)	1.57	(1.18, 2.09)
Clinical Pregnancy	1.72	(1.18, 2.52)	2.68	(1.15, 6.26)	1.65	(0.67, 4.10)	1.54	(1.17, 2.03)
Live birth	1.52	(1.03, 2.25)	2.86	(1.20, 6.81)	1.83	(0.72, 4.67)	1.47	(1.10, 1.95)

[a] Natural log transformation; [b] Generalized linear mixed models; [c] n=316; [d] n=314; [e] Cluster weighted generalized estimating equations; M2: Metaphase 2; RR: Relative risk

Table II.A.4. Regression coefficients and 95% CI for the association of OH-BDEs (ng/g serum) and IVF outcomes from a subset of 215 women (330 IVF cycles) from the EARTH cohort.

	OH-BDEs [a]							
	3-OH47 & 5-OH47		6-OH47		4-OH49		OH-BDE Sum	
Intermediate outcome [b]	B	(95% CI)	B	(95% CI)	B	(95% CI)	B	(95% CI)
Total oocyte yield [c]	-0.01	(-0.10, 0.09)	0.02	(-0.18, 0.22)	0.22	(0.003, 0.44)	0.02	(-0.05, 0.09)
M2 oocyte yield [c]	0.01	(-0.08, 0.11)	0.01	(-0.19, 0.22)	0.26	(0.03, 0.49)	0.03	(-0.04, 0.10)
Endometrial wall thickness [c]	0.21	(-0.25, 0.68)	0.90	(-0.16, 1.95)	0.77	(-0.39, 1.93)	0.31	(-0.05, 0.66)
Fertilization rate [d]	-0.14	(-0.33, 0.05)	0.26	(-0.17, 0.70)	0.002	(-0.49, 0.49)	-0.05	(-0.19, 0.10)
Clinical outcome [e]	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
Implantation	1.28	(1.07, 1.54)	1.56	(1.14, 2.14)	1.28	(0.87, 1.86)	1.22	(1.09, 1.36)
Clinical Pregnancy	1.36	(1.11, 1.67)	1.56	(1.12, 2.18)	1.18	(0.79, 1.77)	1.30	(1.17, 1.44)
Live birth	1.31	(1.03, 1.67)	1.84	(1.26, 2.68)	1.28	(0.80, 2.04)	1.28	(1.10, 1.49)

[a] Natural log transformation; [b] Generalized linear mixed models adjusted for total serum lipid, age, BMI, race (White/Other race), year of OH-BDE sample collection, day 3 FSH levels, IU/L, and IVF protocol (Antagonist, Flare, Luteal phase agonist); [c] n=316; [d] n=314;

[e] Cluster weighted generalized estimating equations adjusted for total serum lipid, age, BMI, race (White/Other), year of BDE sample collection, Day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist); M2: Metaphase 2; RR: Relative risk.

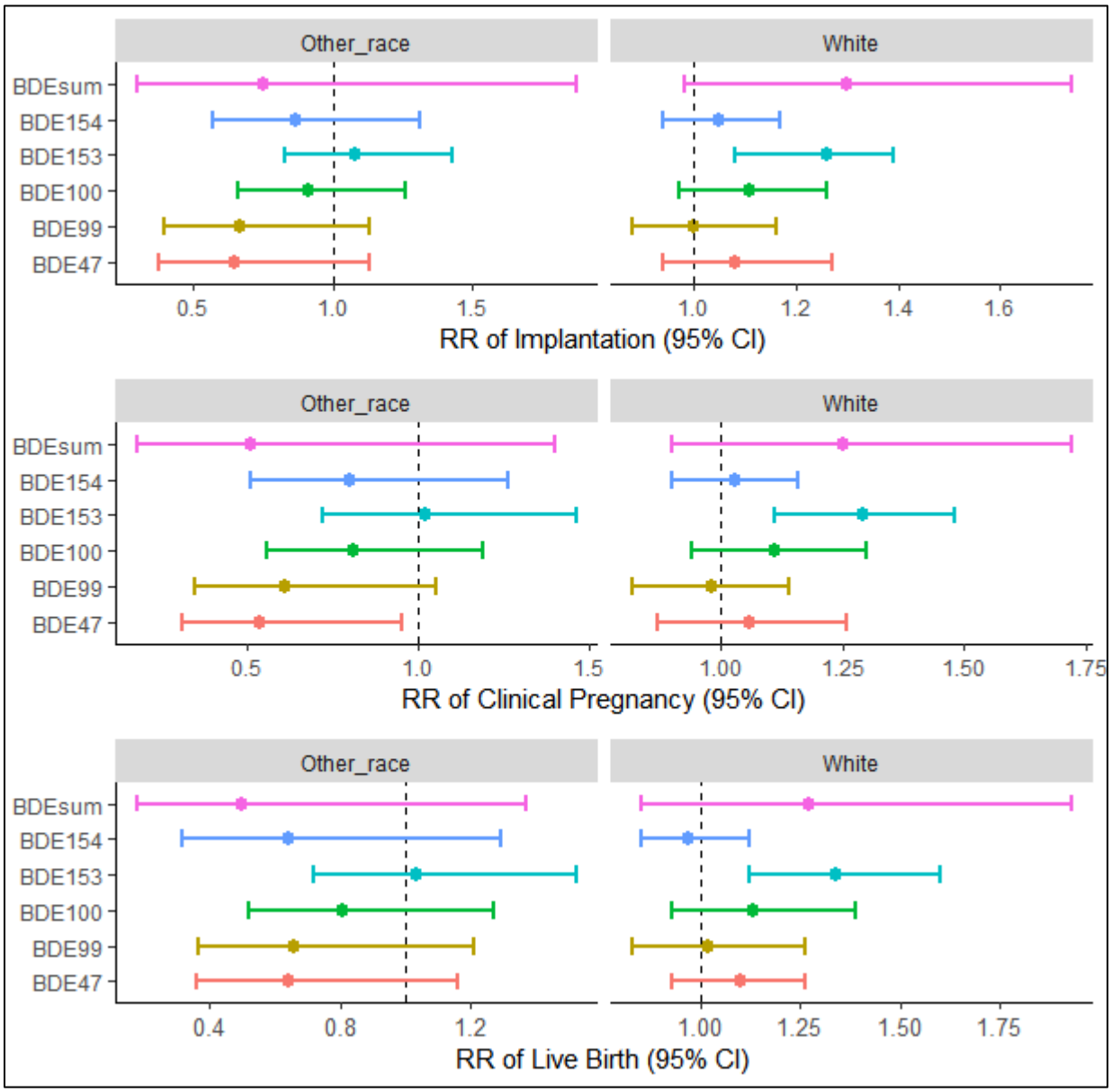
Table II.A.5. Relative risk (95% CI) for the association of PBDE and OH-BDEs (ng/g serum) and clinical IVF outcomes among 215 women from the EARTH cohort stratified by race (White/Other race).

	Clinical IVF outcomes					
	Implantation		Clinical Pregnancy		Live Birth	
	RR	95% CI	RR	95% CI	RR	95% CI
BDE						
BDE47						
White	1.07	(0.95, 1.19)	1.04	(0.91, 1.18)	1.08	(0.92, 1.28)
Other race	0.73	(0.49, 1.09)	0.63	(0.41, 0.96)	0.72	(0.46, 1.12)
BDE99						
White	1.00	(0.89, 1.13)	0.98	(0.86, 1.12)	1.02	(0.86, 1.21)
Other race	0.72	(0.47, 1.11)	0.67	(0.43, 1.04)	0.71	(0.43, 1.17)
BDE100						
White	1.09	(0.97, 1.22)	1.09	(0.95, 1.25)	1.11	(0.94, 1.33)
Other race	0.92	(0.69, 1.22)	0.83	(0.60, 1.16)	0.83	(0.56, 1.23)
BDE153						
White	1.17	(1.06, 1.29)	1.22	(1.09, 1.37)	1.25	(1.09, 1.44)
Other race	1.06	(0.86, 1.32)	1.02	(0.77, 1.36)	1.03	(0.77, 1.39)
BDE154						
White	1.08	(0.91, 1.27)	1.04	(0.85, 1.26)	0.96	(0.78, 1.19)
Other race	0.80	(0.43, 1.52)	0.71	(0.36, 1.42)	0.50	(0.18, 1.47)
BDE Sum						
White	1.25	(0.98, 1.61)	1.21	(0.92, 1.60)	1.24	(0.87, 1.78)
Other race	0.76	(0.35, 1.72)	0.55	(0.23, 1.34)	0.55	(0.23, 1.31)
OH-BDEs						
3-OH47 & 5-OH47						
White	1.32	(1.10, 1.60)	1.39	(1.13, 1.70)	1.38	(1.08, 1.78)
Other race	0.90	(0.55, 1.46)	1.06	(0.59, 1.89)	0.87	(0.49, 1.55)
6-OH47 *						
White	1.55	(1.12, 2.17)	1.59	(1.09, 2.30)	1.71	(1.10, 2.67)
Other race	1.61	(0.56, 4.68)	1.59	(0.42, 5.99)	3.07	(0.70, 13.47)
4-OH49						
White	1.39	(0.94, 2.06)	1.35	(0.88, 2.07)	1.37	(0.83, 2.26)
Other race	0.24	(0.05, 1.20)	0.27	(0.05, 1.45)	0.23	(0.04, 1.29)
OH-BDE Sum						
White	1.25	(1.11, 1.41)	1.28	(1.12, 1.47)	1.29	(1.09, 1.52)
Other race	0.91	(0.64, 1.29)	1.00	(0.66, 1.50)	0.96	(0.63, 1.47)

Models adjusted for total serum lipid, age, BMI, year of BDE/OH-BDE sample collection, day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist). *Results are presented, however

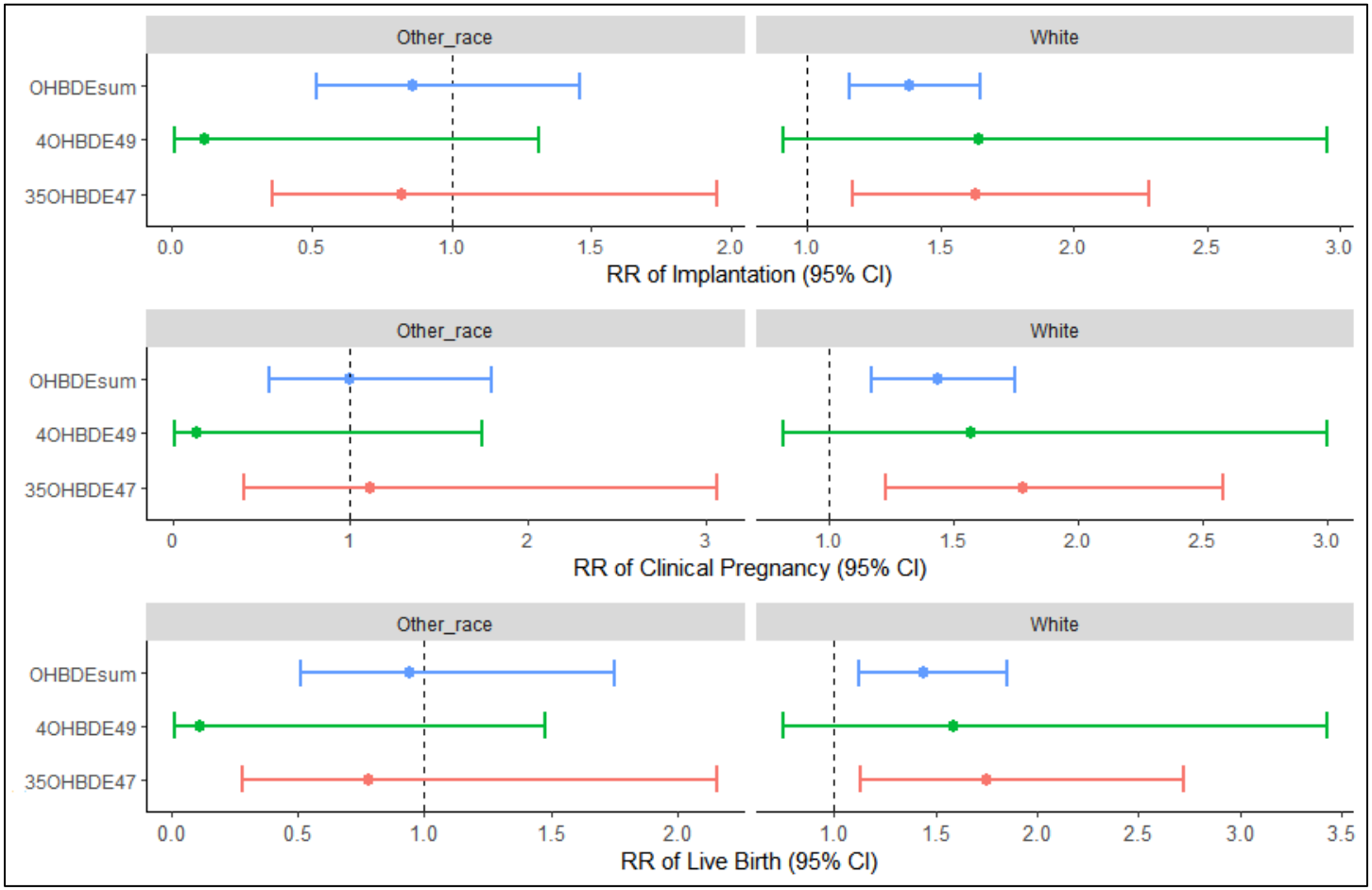
models were unstable and should be interpreted with caution; RR: Relative risk. Successful implantation: White: n= 158, Other race: n= 23, successful clinical pregnancy: White: n= 135, Other race: n= 20, and Live Birth: White: n= 108, Other race: n=16)

Figure II.A.1. Adjusted relative risk (RR) (95% CIs) for clinical outcomes among women with an interquartile range increase in BDE concentrations (ng/g serum) stratified by race (Other race: n=43 IVF cycles or White: n=287 cycles).



Models adjusted for total serum lipid, age, BMI, year of BDE sample collection, day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist). RR: Relative risk.

Figure II.A.2. Adjusted relative risks (RR) (95% CIs) for clinical outcomes among women with an interquartile range increase in OH-BDE metabolite concentrations (ng/g serum) stratified by race (Other race: n=43 IVF cycles or White: n=287 cycles).



Models adjusted for total serum lipid, age, BMI, year of BDE/OH-BDE sample collection, day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist). Results for 6-OH-BDE47 were omitted due to model instability. RR: Relative risk

Figure II.A.3. BDE concentration (ng/g lipid) stratified by race (Other race/White).

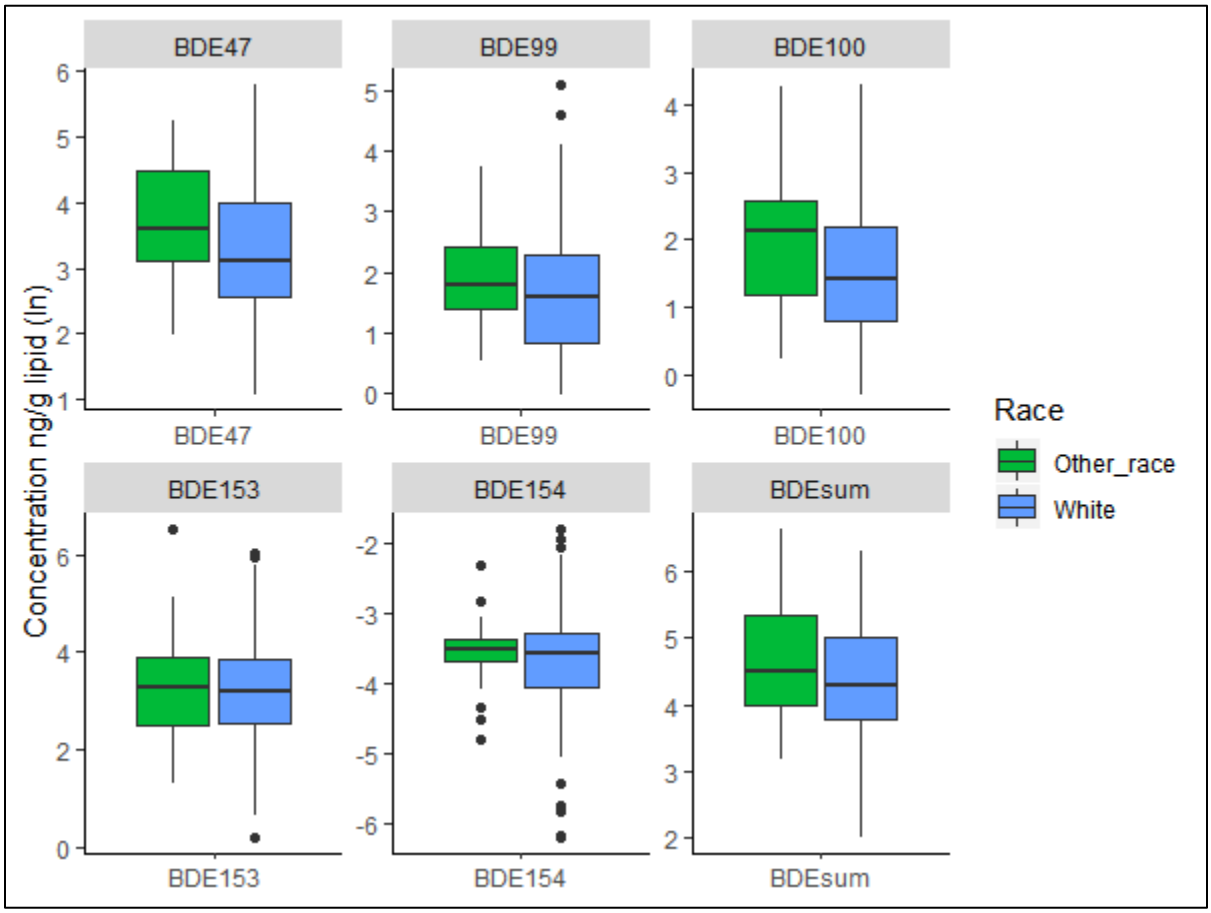
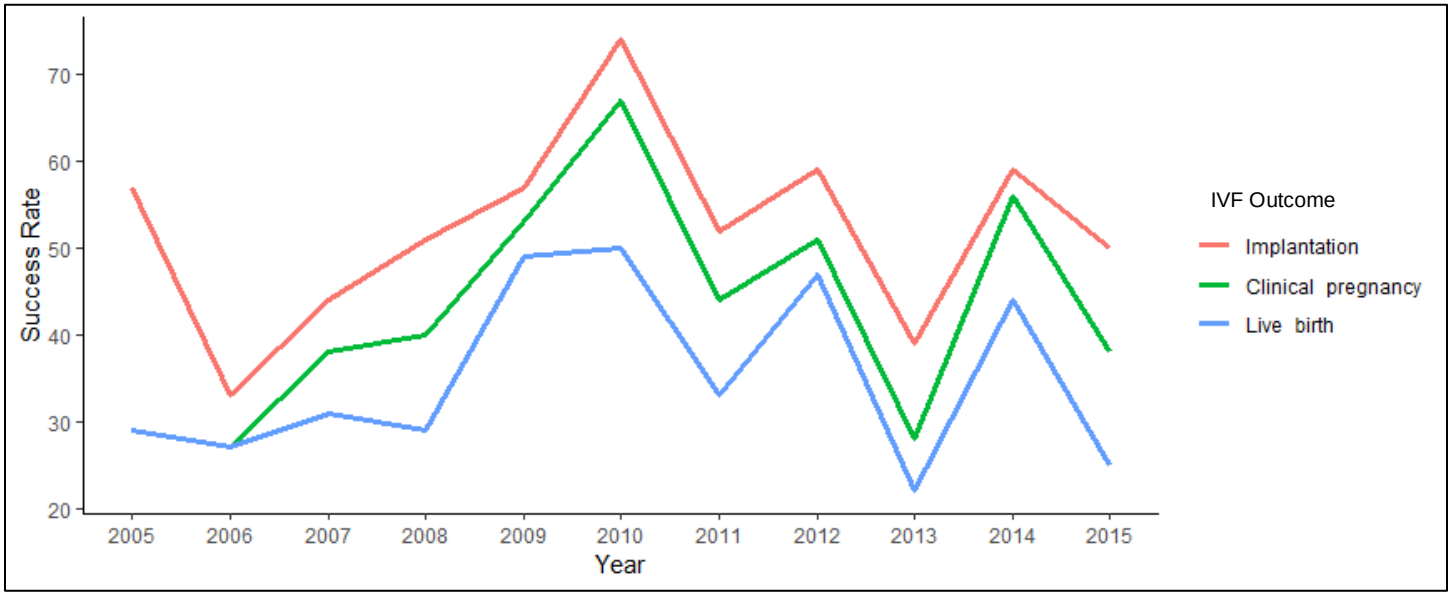


Figure II.A.4. Clinical IVF outcome success rates from 215 women from the EARTH cohort by year (2005-2015) of BDE and OH-BDE sample collection.



Only one sample was collected in 2016 and not included

Chapter III

AIM 2: Reproductive Outcomes Associated with Serum Polybrominated Diphenyl Ether and Hydroxylated Brominated Diphenyl Ether Concentrations Among Couples Seeking Fertility Treatment: A Paternal Perspective

Abstract

Background: Polybrominated diphenyl ethers (PBDEs) have been phased out of production for nearly a decade yet are still frequently detected in serum of U.S. adults. PBDE concentrations in women have been associated with adverse reproductive outcomes and laboratory studies suggest hydroxylated-BDEs (OH-BDEs) may act as endocrine disruptors. However, studies investigating the associations of male PBDE and OH-BDE exposure with female partners pregnancy outcomes are lacking. Our study contributes to a limited literature by assessing 1) the joint effects of paternal and maternal serum PBDE concentrations on pregnancy outcomes and 2) the association between paternal serum OH-BDE concentrations and pregnancy outcomes.

Methods: This analysis included 189 couples (contributing 285 in vitro fertilization (IVF) cycles) recruited between 2006-2016 from a longitudinal cohort based at Massachusetts General Hospital Fertility Center who completed at least one IVF cycle and had an available blood sample at study entry. Five PBDE congeners (47, 99, 100, 153, and 154) and four OH-BDEs (3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, and 4-OH-BDE49) were quantified in serum. Log-transformed PBDEs and OH-BDEs were modeled in

quartiles for associations with fertilization, implantation, clinical pregnancy, and live birth using multivariable generalized mixed models and cluster weighted generalized estimating equations while adjusting for male and female lipids, age, body mass index, year of serum sample collection, and infertility diagnosis (female, male, unknown).

Results: Congeners 47, 153, 154, and metabolites 6-OH-BDE47 and 4-OH-BDE49 were frequently detected (84% >method detection limit (MDL)). Lipid-adjusted concentrations of PBDEs and OH-BDEs were higher in females than in male partners. There were no clear patterns of increases in risk of adverse IVF outcomes associated with PBDEs and OH-BDEs. However, some decreases in associations with pregnancy outcomes (implantation, clinical pregnancy, and live birth) were observed in isolated quartiles; for example, quartile 2 of BDE99 and BD100, and quartile 3 of BDE153.

Conclusion: Overall, male serum PBDEs or OH-BDES were not associated with couples' reproductive outcomes, although some specific quartiles were associated with decreased probabilities of implantation, clinical pregnancy, and live birth. Our assessment of couple level exposure is unique compared to the current literature and highlights the importance of including male and female exposures in the assessment of the influence of environmental toxicants on pregnancy outcomes.

Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants which have been phased out of production for nearly a decade, yet concentrations among US adults remain high [1]. The most frequently detected congeners (47, 99, 100, 153, and 154) are found in (but not exclusively) the PentaBDE commercial mixture, which was primarily manufactured in the US until the voluntary phase out at the end of 2004 [2,3]. PBDEs are not covalently bound but physically combined with materials, which allows them to leach into surrounding environments and bioaccumulate in adipose tissues, with half-lives ranging from weeks to years [4]. PBDEs were added to consumer products including upholstered furniture, carpeting, electronics, and plastics as flame retardants. In the US, PBDE exposure is predominantly from dust due to its common use in these products, while diet is a main source of exposure in European countries where it was less commonly used [3]. Hydroxylated-BDEs (OH-BDEs), products of oxidative metabolism from PBDEs in mammals, also bioaccumulate in humans and have been detected in adult serum [5]. Some OH-BDEs are of a natural origin (e.g. biogenic from marine sponges) and may contribute to exposure via seafood diets [6].

PBDE exposure has been associated with adverse reproductive health outcomes in both men and women. Elevated concentrations of BDE99 and BDE153 in women have been associated with failed implantation and longer time to pregnancy (TTP) [7,8]. Congeners 47, 100, 153, and 154 have also been associated with altered hormone levels and poorer semen quality in men, while low-dose *in utero* exposure to BDE99 in mice was associated with poor semen quality in male offspring [9–12]. However,

laboratory studies suggest OH-BDEs may act as endocrine disruptors and have greater toxic effects compared to PBDEs [13,14].

We have previously reported that serum PBDE and OH-BDE concentrations among women had unexpected positive associations with implantation, clinical pregnancy, and live birth (*Ingle, et al. In press).* However, considerations for male exposure with fertility and pregnancy outcomes are pivotal in understanding the comprehensive impact of PBDE exposure on reproductive health. This analysis examines the relationship of couple (male and female) PBDE and OH-BDE exposures with fertility metrics and pregnancy outcomes.

Methods

Study Population

The male participants (n=189) and their female partners (n=189) included in our analysis were a subset of study participants recruited between 2006-2016 from the EARTH study, an ongoing longitudinal prospective pre-conception cohort study of the environment, dietary, and lifestyle impacts on reproductive health [15]. Men (18-55 years) and women (18-46 years) were recruited from Massachusetts General Hospital (MGH) Fertility Center. Approximately 60% of participants contacted by research staff participated in the study [16]. Couples (male and female) must have contributed their own gametes, completed at least one *in vitro* fertilization (IVF) cycle (n=285 cycles), and provided a blood sample for flame retardant and metabolite quantification. At study entry, research staff collected demographic data and pregnancy history. Research protocols were approved by the Ethics and Research Committees of MGH, Harvard T.H. Chan School of Public Health, University of Michigan, and Duke University. The

study was described in detail to participants, all questions were answered, and informed consent was obtained from all participants.

PBDE and OH-BDE Collection and Measurement

PBDE and metabolite protocols have been described in detail elsewhere (*Ingle, et al. In press).* Briefly, 5 mL blood samples collected at study entry were aliquoted, frozen, and stored (-80° C) before shipment overnight to Dr. Stapleton's laboratory at Duke University (Durham, NC). Five PBDE congeners: 47, 99, 100, 153, and 154 and four OH-BDE metabolites: 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47, and 4-OH-BDE49 were quantified in serum.

Samples were weighed and spiked with internal standards (monofluorinated BDE 69, 13C BDE 209, and 13C-6-OH-BDE47). Serum was diluted with water and formic acid before solid phase extraction (SPE) (Oasis HLB, Waters Corp.). Dichloromethane (DCM) and ethyl acetate (50:50) removed both PBDES and OH-BDEs from the SPE column. Samples were dried, rejuvenated with hexane (1 mL) and extract cleaning via a 1.0 g silica column. PBDEs were removed with 10 mL of hexane and OH-BDEs with 10 mL of DCM hexane solution. PBDEs were analyzed using gas chromatography negative chemical ionization mass spectrometry (GC/ECNI-MS) and OH-BDEs were measured using liquid chromatography tandem mass spectrometry (LC/MS-MS) [17]. Accuracy was verified by extracting a human serum Standard Reference Material (SRM 1957) from the National Institute of Standards and Technology (NIST). Measured values were between 73%- 97% of the certified values. Total lipids were derived from total serum cholesterol and triglycerides using the following formula: TL (g/l) = [(TC x 1.12) + (TG x 1.33) + 1.48 where TL= total lipids, TG= serum triglycerides, and TC=

serum cholesterol (Covaci *et al.*, 2006). Missing total lipids (males n=4 and females n=13) were replaced with the median (males= 628.6 and females=509.15).

Clinical Protocols and Outcomes

Clinical staff collected participants' date of birth and measured height and weight to calculate body mass index (BMI, kg/m^2) at study entry. At the beginning of each cycle, clinical data are abstracted from the female partners' electronic health records by research staff. Clinical protocols and outcomes have previously been described [18]. Briefly, initial infertility diagnoses are given by a physician at MGH Fertility Centers in accordance with the Society for Assisted Reproductive Technology (SART) definitions [19,20]. Depending on infertility evaluation and other clinical factors, one of three ovarian stimulation protocols was selected: (1) luteal phase gonadotrophin releasing hormone (GnRH) agonist, (2) follicular phase GnRH agonist or "flare" stimulation, or (3) GnRH antagonist. Fertilization was confirmed 17-20 hours after IVF or intracytoplasmic sperm injection (ICSI) by the presence of an oocyte with two pronuclei. Fertilization rate was defined as the number of two pronuclear embryos divided by the number of metaphase II (M2) oocytes. Successful implantation was confirmed when serum beta human chorionic gonadotropin (β-hCG) levels were > 6 mIU/mL, approximately 17 days after egg retrieval. Implantation was characterized as the presence of an intrauterine pregnancy confirmed by ultrasound and elevated β-hCG levels (approximately 6 weeks gestation). Live birth was defined as the birth of a neonate at or after 24 weeks gestation.

Statistical Analysis

Demographic characteristics for males were characterized using medians together with interquartile ranges (IQRs) for continuous variables, and frequencies together with percentages for categorical variables. PBDEs and OH-BDE concentrations below method detection limit (MDL) were imputed to MDL/√2 (Hornung and Reed, 1990). Unadjusted and lipid-adjusted congeners and metabolites were described using geometric means (GM), 95% confidence intervals (CIs), and selected percentiles. Spearman correlation coefficients were used to assess relationships between male and female serum PBDE and OH-BDE concentrations. Distributions of congeners and metabolites were right-skewed and transformed by the natural logarithm (ln).

Concentrations of PBDEs and OH-BDEs were evaluated individually and summed. Concentrations were divided into quartiles before inclusion in regression models to account for possible non-linear relationships. The p-value for trend (P-trend) was calculated from a regression model including the median ln-transformed PBDE or OH-BDE concentration of each quartile. Associations of congeners and metabolites with fertilization rate were evaluated using multivariable generalized linear mixed models (binomial distribution and logit function), where a random intercept is introduced to account for multiple cycles per couple. Associations with PBDEs and OH-BDES with implantation, clinical pregnancy, and live birth were assessed using cluster weighted generalized estimating equation (CWGEE) models, where the weight was the inverse of the total number of cycles (cluster size) (Williamson, John et al., 2003). CWGEE models have provided more flexibility to allow for more complex within-cluster correlations, and they only need the mean model to be correctly specified. Interpretation in CWGEE is at population level, while in contrast, interpretation in

generalized linear mixed models is at individual level ([23], *Ingle, et al. In press*). Due to some co-elution with a few batches of 3-OH-BDE47 and 5-OH-BDE47 among female samples, male and female metabolites 3-OH-BDE47 and 5-OH-BDE47 were modeled as a sum 3&5-OH-BDE47 (Ingle, et al. In press).

Demographic covariates considered for final models were male and female: total lipids, age and BMI, and male: race (White/Other), education (high school/some college, college graduate, graduate degree), smoking status (never/ever) and year of serum sample collection. Reproductive characteristics considered were history of prior pregnancy, initial infertility diagnosis (female factor, male factor, or unexplained), previous intrauterine insemination (IUI) (yes/no), previous IVF (yes/no), treatment protocol (antagonist, flare, or luteal phase agonist), and ICSI (yes/no). Final covariates were included if they were associated with PBDE concentrations in our cohort, associated with PBDEs based on prior studies, and known to be a predictor of IVF outcomes (Harley et al., 2010b; Johnson et al., 2012). Analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and R version 3.3.5. P-values < 0.05 were considered statistically significant.

Results

Men (n=189) were primarily White (86%), non-smokers (68%) in their mid-thirties (median= 36 yrs.) with a BMI typical of the U.S. population (median= 27 kg/m^2), and they had similar demographic characteristics to a previously reported sample of men from the EARTH cohort (Ingle *et al.*, 2018) (Supplemental Table III.A.1). Almost all men (93%) held at least a college degree. Demographic and reproductive characteristics of female partners (n=189) have been described elsewhere (*Ingle, et al. In press*). Briefly,

female partners were mostly White (86%) with a normal BMI (median=23 kg/m^2) in their mid-thirties (median=35 years). Over half of the women (61%) held a graduate degree and few reported ever smoking (28%). The most common infertility diagnosis was male factor (36%) followed by unexplained infertility (35%) and female factor (29%). The majority of women underwent luteal phase agonist protocol (69%), followed by flare (18%), and antagonist (13%). Over half of fertilization was via ICSI (52%).

Descriptive statistics of unadjusted and lipid-adjusted male PBDEs and OH-BDEs are presented in Table III.1. Congeners 47, 153, and 154 were frequently detected (88% >MDL). Concentrations of BDE47 (GM=12.8 ng/g serum) and BDE153 (GM=14.5 ng/g serum) were over 4-fold higher than BDE99 (GM=2.2 ng/g serum) and BDE100 (GM=2.6 ng/g serum). Metabolites 3-OH-BDE47 and 4-OH-BDE49 were also frequently detected in men (84%>MDL). Median concentrations of 3-OH-BDE47 (0.14 ng/g serum) were more than double those of 6-OH-BDE47 (median=0.06 ng/g serum). Female partners' distributions of serum PBDE and OH-BDE concentrations have previously been described (*Ingle, et al. In press*). Briefly, PBDEs 47, 100, 153, and metabolites 3-OH-BDE47, and 4-OH-BDE49 were frequently detected (70%>MDL) and concentrations of BDE47 and BDE153 were approximately 5-fold higher than BDE99 and BDE100. Median concentrations of 3-OH-BDE47 (0.12 ng/g serum) in women were slightly higher than 4-OH-BDE49 (median=0.09 ng/g serum) concentrations. The median of lipid-adjusted concentrations of all congeners were higher in female partners compared to males ($p<0.001$) (Figure III.1). Lipid adjusted median concentrations of BDE47 (24.6 ng/g lipid) and BDE153 (24.5 ng/g lipid) were almost 3-fold higher among female partners compared to males (8.1 and 7.7 ng/g lipid). OH-BDE concentrations

were also higher in female partners compared to males ($p<0.001$) (Supplemental Figure III.A.1). Median concentrations of 4-OH-BDE49 (0.21 ng/g lipid) and 6-OH-BDE47 (0.18 ng/g lipid) were nearly three and four times (respectively) higher among female partners compared to men (0.07 and 0.04 ng/g lipid) (Supplemental Figure III.A.1).

Correlations of serum PBDEs and OH-BDEs for male and female partners are presented in Figure III.2. Among men, correlations for congeners 47, 99, and 100 were the strongest (r: 0.84-0.85). Correlations for BDE153 and BDE154 were slightly weaker (r: 0.38-0.54), yet still statistically significant. Metabolites 6-OH-BDE47 and 4-OH-BDE49 were strongly correlated (r=0.64). The combined metabolite 3&5-OH-BDE47 was moderately correlated with BDEs 47, 99, and 100 (r: 0.51-0.61). A detailed description of PBDE and OH-BDE correlations among female partners has been described elsewhere (*Ingle, et al. In press*). Correlations for PBDES between females and males were strongest for BDE47 (r=0.47) and weakest for BDE153 (r=0.24). Correlations for OH-BDEs between males and their female partners were weak (r: 0.22-0.34) but statistically significant, except for the association of female 3&5-OH-BDE47 and male 6-OH-BDE47 ($p>0.05$). PBDE and OH-BDE concentrations among men declined between 2006 and 2007 and remained consistent throughout the study (Figure III.3). The largest decline was observed for BDE47 which decreased 84% between 2006-2007. Metabolite concentrations fluctuated, yet generally declined over the study period.

No associations were observed between any PBDEs or OH-BDEs and fertilization rate (Tables III.A.2 and III.A.3). However, some of the quartiles, specifically quartile 2 (Q2) or Q3 were associated with poorer IVF outcomes. For instance, a 38% decrease

in the probability of implantation (Relative risk (RR)=0.62; 95% CI: 0.45, 0.84; p=0.002), 39% decrease in probability of clinical pregnancy (RR=0.61; 95% CI: 0.42, 0.86; p=0.01), and 36% decrease in the probability of live birth (RR=0.64; 95% CI: 0.44, 0.91; p=0.02) was observed among female partners of men in Q2 of serum BDE99 concentrations compared to Q1 (reference group) (Figure III.4). Similarly, a decrease in the probability of implantation, clinical pregnancy, and live birth was found among female partners of men in Q2 of serum BDE100 concentrations compared to Q1 (RR=0.62; 95% CI: 0.45, 0.86; p=0.004, RR=0.59; 95% CI: 0.41, 0.84, p=0.004, and RR=0.56; 95% CI: 0.37, 0.87; p=0.01, respectively). On the other hand, Q3 serum concentrations of BDE153 were associated with a 37% decrease in the probability of implantation (RR=0.63; 95% CI: 0.46, 0.86; p=0.004), 34% decrease in the probability of clinical pregnancy (RR=0.64; 95% CI: 0.47, 0.92; p=0.02), and 38% decrease in the probability of live birth (RR=0.62; 95% CI: 0.40, 0.96; p=0.03) compared to men in Q1 group of exposure. A 37% increase in the probability of implantation was observed for female partners of men in Q2 of serum BDE154 concentrations compared to men in Q1 (RR=0.63; 95% CI: 1.01, 1.82; p=0.04). Summed serum OH-BDE concentrations in Q2 and Q4 among males were associated with an increase in the probability of live birth for female partners (RR=2.17; 95% CI: 1.34, 3.53; p=0.001 and RR=2.12; 95% CI: 1.29, 3.49; p=0.003; p-trend=0.03, respectively) compared to men with summed serum concentrations in Q1 (Figure III.5).

Discussion

This study investigated associations of paternal serum concentrations of PBDEs and OH-BDEs with couples' pregnancy outcomes while accounting for female exposure.

While we found overall no significant associations, we also observed decreased probabilities of successful implantation, clinical pregnancy, and live birth among female partners of men in Q2 of serum BDE99 and BDE100 concentrations, and Q3 concentrations of BDE153, compared to men in Q1. These non-linear relationships were unexpected, and possibly spurious, yet biologically plausible if PBDEs are acting as endocrine disruptors which have been associated with unconventional dose-response relationships [24,25]. For instance, non-linear dose-response relationships have been observed between congeners 99, 100, and 153 thyroid stimulating hormone levels during pregnancy [26].

Several studies have observed negative associations between sperm count, concentration, and morphology and BDE153 in men, while low doses of BDE99 in mice have been associated with decreased sperm and spermatid counts [9,11,27,28] . Other studies suggest PBDEs act as an endocrine disruptor and alter male reproductive hormones. Congeners 47 and 99 have been inversely associated with inhibin B and positively associated with follicular stimulating hormone (FSH) and lower levels of inhibin B and higher levels of FSH are often observed in subfertile men [12]. Reported associations between PBDEs and thyroid functions is inconsistent [29–31]. However, both hypo and hyperthyroidism have been associated with male infertility [32,33].

To the best of our knowledge, only one prior study has assessed the relationship of serum PBDE concentrations among couples with TTP and we are the first to evaluate pregnancy outcomes from conception to live birth. A prior study of 501 couples found no associations with the pentaBDEs and TTP yet did observe a 14% decrease in

fecundability odds ratio (FOR) with elevated male serum BDE183 concentrations [34]. However, there was no adjustment for female concentrations or covariates.

Couple comparison studies of PBDEs and OH-BDEs are limited. However, adjusted GMs for PBDEs were significantly higher overall in males compared to females in a pooled sample from the National Health and Nutrition Examinations Survey (NHANES) [1]. Correlations for BDE153 were the weakest among our couples (r=0.24) and possibly a result of exposure through diet which can vary by individual [35,36]. Differences among couples could also be a result of separate 'workday' microenvironments. In a sample of 20 homes in Boston, MA, congeners from the PentaBDE mixture were 72% higher in the main living area compared to the bedroom [37]. Lower PentaBDE dust concentrations have also been observed in recently constructed buildings compared to older buildings in the Boston area [38].

However, we observed more comparable distributions among women and their partners for unadjusted PBDE and OH-BDE concentrations and therefore serum lipid levels may be driving the difference in lipid-adjusted concentrations. We observed statically higher total serum lipids and BMIs in men compared to women (Figure III.A.2). PBDEs bioaccumulate in adipose tissue and restrict exposure to vital organs, however adipose storage and lipid metabolism varies by sex [39]. Therefore, it is possible that lipid-adjusted concentrations were higher in females due to sex differences in lipid metabolism and fat storage. However, differences in PBDE concentrations could be attributed to BMI as an inverse association was also observed for congeners 47 and 153 with BMI among a sample of women from CA [41]. A laboratory study also observed impaired glucose homeostasis in lean mice undergoing IVF compared to obese mice

exposed to polychlorinated biphenyls (PCBs) through diet [40]. Therefore, it is also possible that IVF alters the rate at which lipids are metabolized and results in increased PBDE concentrations circulating throughout females.

Serum concentrations of congeners 47, 99, 100, and 153 among our men are similar to those seen in another male cohort (n=50) in Boston [42]. As far as we are aware, we are the first study to describe OH-BDEs among adult men in the US. Comparisons of PBDE and OH-BDE concentrations of our female partners with other studies has been described elsewhere (*Ingle, et al., In press*). Briefly, PBDE concentrations were higher in our female partners compared to women in other cohorts. Concentrations of BDE47 among our sample were approximately double those from women in California and North Carolina, yet similar for BDE99 [7,17]. BDE153 concentrations among our female partners were also higher compared to a sample of women in Canada [44]. Concentrations of 6-OH-BDE47 were higher compared to pregnant women in NC, yet lower than pregnant women in IN [5,17]. Concentrations of 3-OH-BDE47 and 4-OH-BDE49 were higher in our female partners compared to pregnant women in IN. Higher concentrations among our women could possibly be a result of rapid lipid peroxidation in women undergoing IVF compared to women in the general population which would result in the release of chemicals stored in adipose tissues. [45].

Detection rates were high for BDEs: 47, 153, and 154 and OH-BDEs 3-OH-BDE47 and 4-OH-BDE49 (84% >MDL) in males, yet concentrations subtly declined over the 10-year study period. We observed the largest decrease in serum PBDE concentrations between 2006-2007 which is inconsistent with a previous EARTH study as well as cohorts in California which observed substantial declines in concentrations over time

([46],*Ingle, et al., In press)*. Such a drastic decrease in the early years of the study was likely a result of the phase-out of PBDEs. Although the PentaBDE phase-out was not mandatory until 2005, many manufacturers voluntarily began to restrict these compounds a few years prior.

Although we observed statistically significant associations with several congeners and pregnancy outcomes for some exposure quartiles, a larger sample size would increase study power. Type l error is also plausible as we compared many congeners and metabolites with many outcomes. While IVF cohorts may not be as generalizable compared to TTP studies, our results are generalizable to other subfertile cohorts and possibly the general population if the biological response to PBDEs is similar for both IVF and non-IVF patients. Nevertheless, our cohort of IVF couples represents a susceptible population. Prospective pre-conception studies allow for the highlighting of specific critical windows during conception and gestation [46]. Our study design expands upon other pre-conception cohorts by measuring early developmental and clinical endpoints which are not observable in studies of the general population. However, if PBDEs and OH-BDES do elicit responses through endocrine disruption, the addition of a reproductive hormone analysis could establish a biological relationship between PBDEs and OH-BDEs with pregnancy outcomes. Although typically a homogeneous population, IVF patients are highly motivated and require multiple office visits which maximize participant retention rate. Finally, infertility is a couple-based disease, and inclusion of couples allows for a more comprehensive assessment of the impact of PBDE and OH-BDEs with couple-based outcomes.

Conclusion

Despite a decade long phase-out, PBDEs were still widely detected and serum concentrations of PBDEs, on a lipid adjusted basis, were significantly higher in female compared to male partners. Overall, we did not observe any associations between male serum PBDEs or OH-BDEs and pregnancy outcomes, although some specific quartiles were associated with decreased probabilities of implantation, clinical pregnancy, and liver birth. Our assessment of couple level exposure is unique compared to the current literature and highlights the importance of including male and female exposures in the assessment of environmental toxicants with pregnancy outcomes.

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Table III.1. Distribution of unadjusted and lipid adjusted PBDEs and OH-BDEs among 189 men from the EARTH cohort.

					Percentiles				
PBDEs	N>MDL	(%)	GM	(95% CI)	25th	50th	75th	95th	Max
Unadjusted (pg/g serum)									
BDE47	166	87.8	12.8	(11.0, 15.0)	5.7	12.1	25.5	103.2	374.3
BDE99	109	57.7	2.2	(1.9, 2.5)	<MDL	1.6	3.7	14.7	37.8
BDE100	112	59.3	2.6	(2.3, 3.0)	<MDL	2.0	4.6	14.6	78.9
BDE153	181	95.8	14.4	(12.2, 16.9)	6.5	10.8	26.8	146.1	768.0
BDE154	196	96.3	3.7	(3.2, 4.1)	2.2	3.7	6.3	14.3	32.8
Total BDEs			43.6	(38.2, 49.8)	22.6	36.8	74.6	238.4	808.0
Lipid Adjusted (ng/g lipid)									
BDE47			8.8	(7.1, 9.9)	3.5	8.1	17.7	60.7	224.5
BDE99			1.4	(1.2, 1.6)	0.79	1.1	2.4	8.9	46.1
BDE100			1.7	(1.5, 2.0)	0.96	1.3	3.1	9.2	47.3
BDE153			9.4	(7.9, 11.1)	4.2	7.7	18.1	94.0	530.7
BDE154			2.4	(2.1, 2.7)	1.3	2.4	4.3	12.1	18.9
Total BDEs			28.5	(24.8, 32.9)	14.5	25.4	47.1	150.0	558.4
OH-BDEs									
Unadjusted (pg/g serum)									
3-OH-BDE47	159	84.1	0.15	(0.13, 0.17)	0.09	0.14	0.27	0.74	1.3
5-OH-BDE-47	13	4.6	0.02	(0.02, 0.02)	0.01	0.02	0.02	0.03	0.46
6-OH-BDE47	107	56.6	0.07	(0.06, 0.08)	<MDL	0.06	0.18	1.03	2.3
4-OH-BDE49	166	87.8	0.10	(0.08, 0.12)	0.04	0.10	0.24	1.1	2.1
Total OH-BDEs			0.40	(0.35, 0.46)	0.19	0.34	0.73	2.2	4.4
Lipid-Adjusted (ng/g lipid)									
3-OH-BDE47			0.10	(0.08, 0.11)	0.05	0.09	0.17	0.51	1.8
5'OH-BDE-47			0.01	(0.01, 0.01)	0.01	0.01	0.02	0.03	0.39
6'OH-BDE47			0.05	(0.04, 0.06)	0.02	0.04	0.12	0.66	1.6
4'OH-BDE49			0.07	(0.05, 0.08)	0.02	0.07	0.17	0.75	1.6
Total OH-BDEs			0.26	(0.23, 0.30)	0.12	0.23	0.54	1.5	3.4

MDL: Method detection limit; GM: Geometric mean; CI: Confidence interval

Figure III.1. Distributions of serum PBDEs concentrations (ng/g lipid) among 189 couples from the EARTH study.

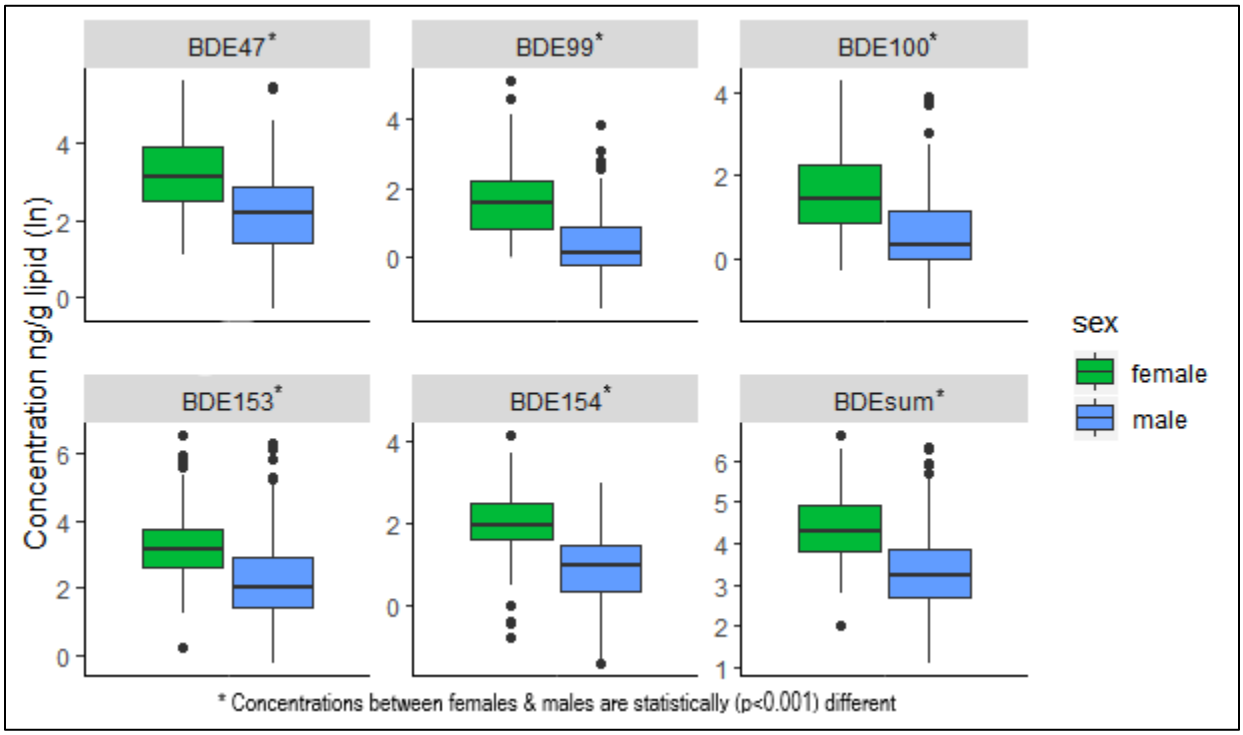


Figure III.2. Spearman correlation coefficients for serum concentrations of PBDEs and OHBDEs (ng/g lipid) among 189 couples from the EARTH study.

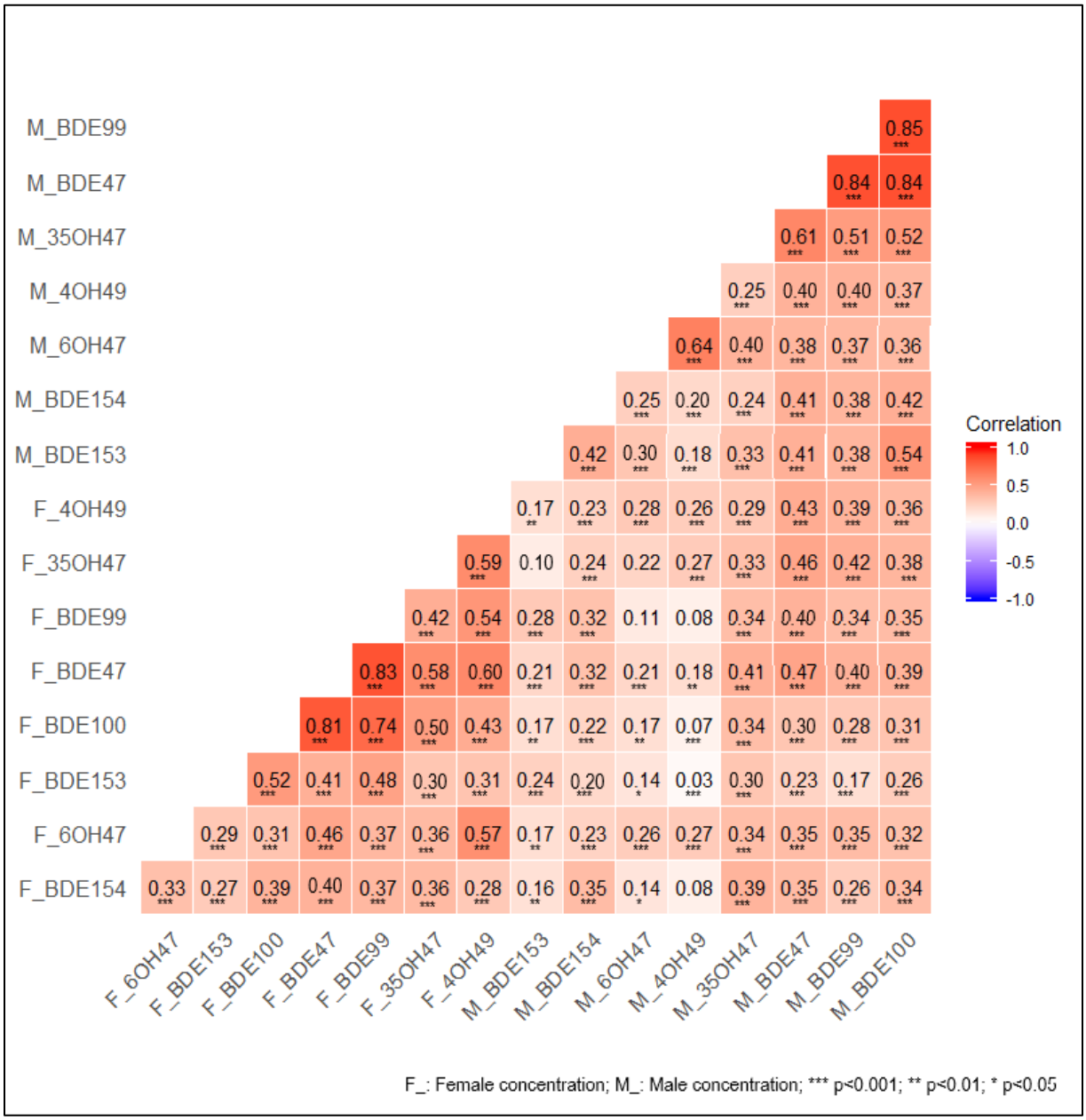
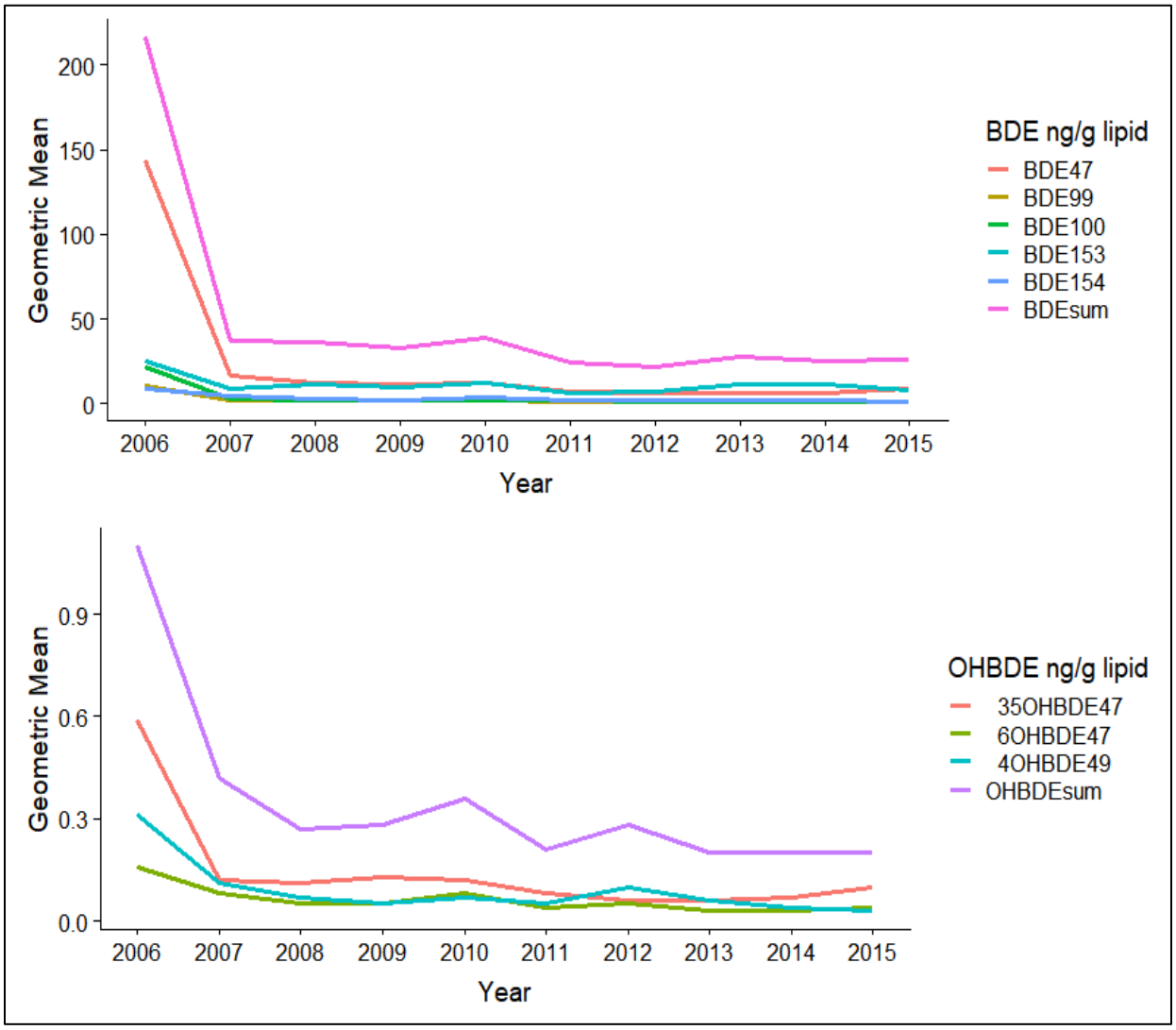
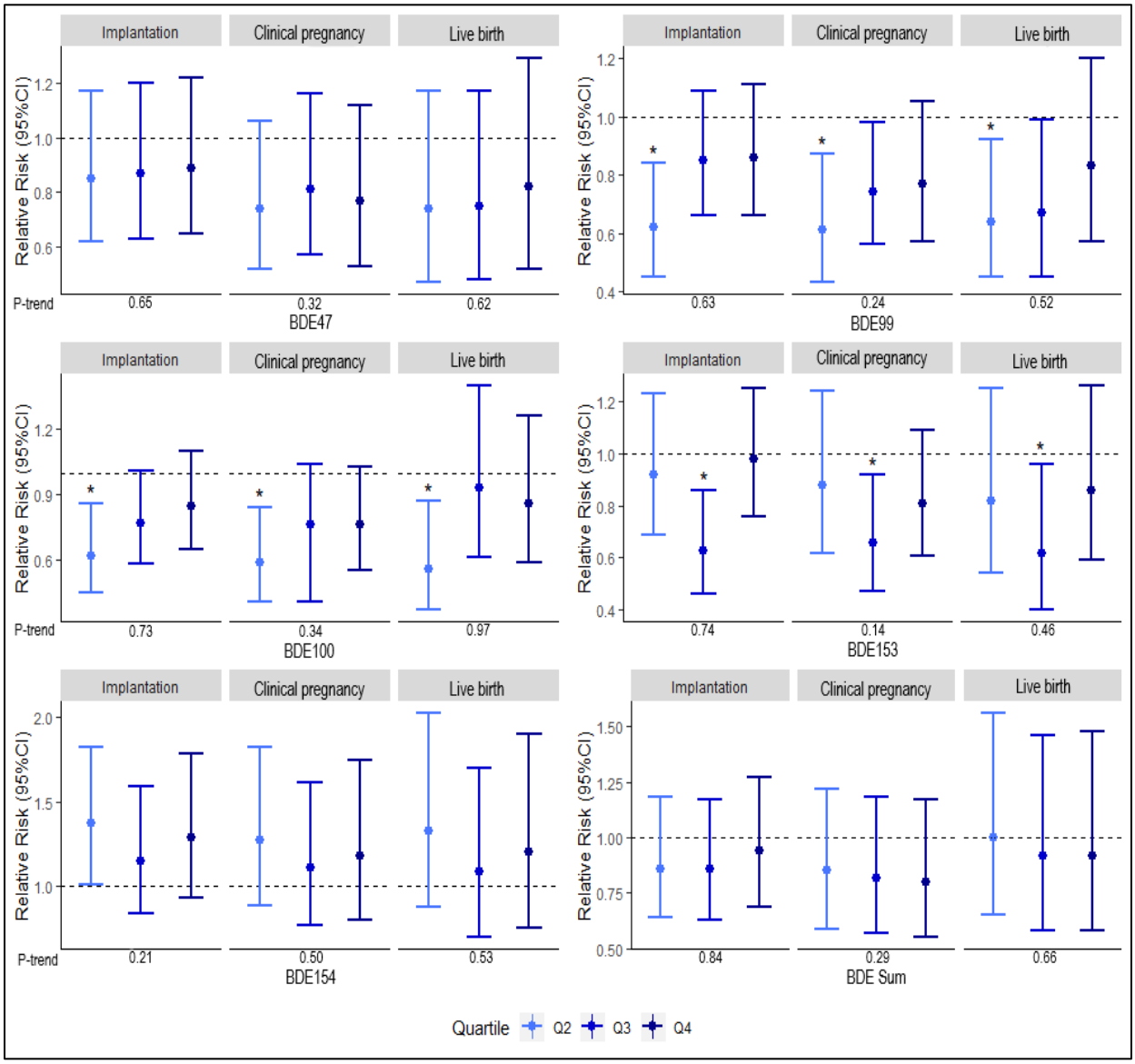


Figure III.3. Geometric means of serum PBDE and OH-BDE concentrations among 189 men from the EARTH cohort by year.



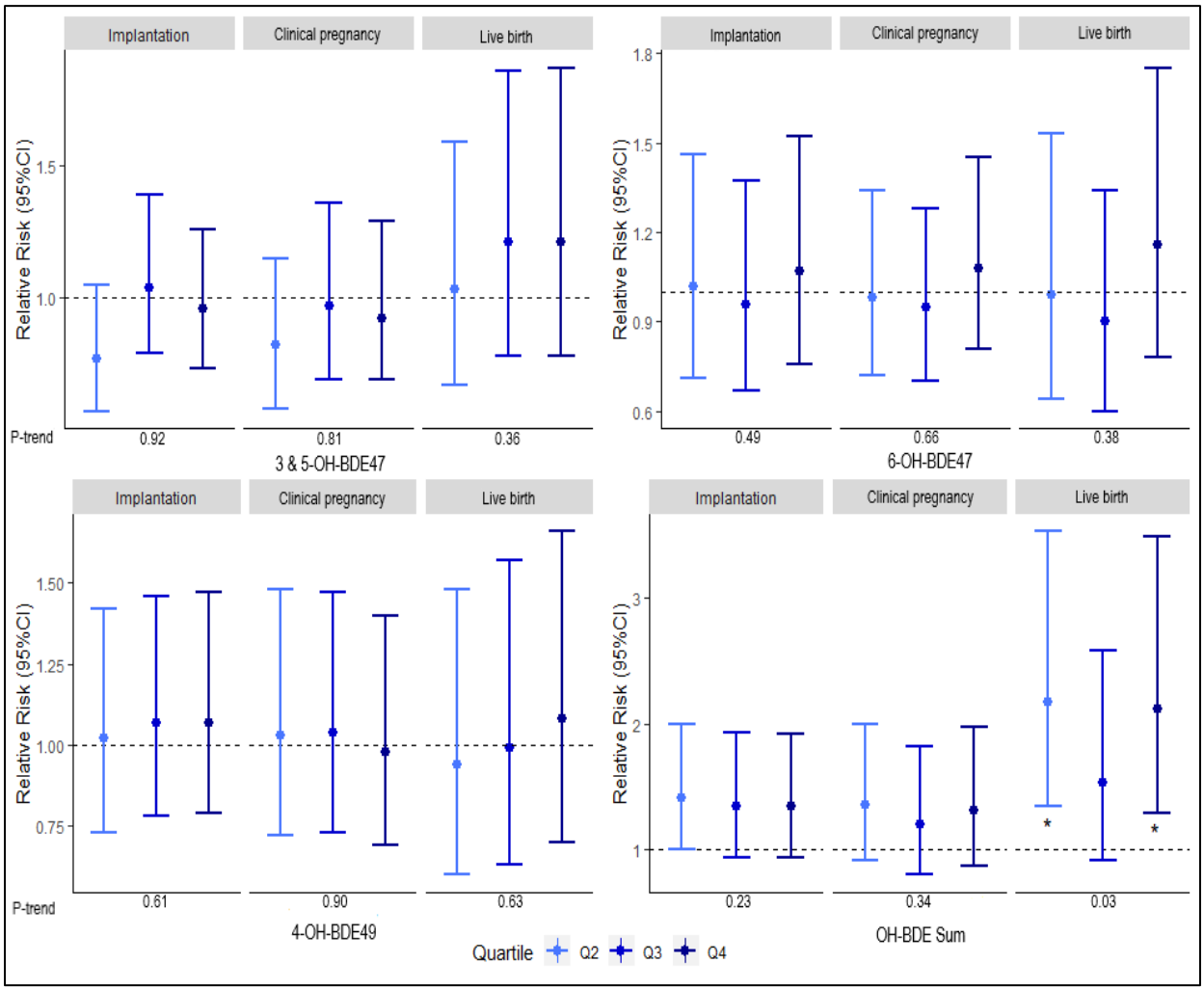
2006: n=2; 2007: n=5; 2008: n=26; 2009: n=44; 2010: n=45; 2011: n=48; 2012: n=38; 2013: n=36; 2014: n=32; 2015: n=8; 2016: Only one sample collected and not included.

Figure III.4. Relative risk (95% CI) of clinical IVF outcomes by quartile of serum PBDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.



Cluster weighted generalized estimating equations adjusted for female serum PBDE concentrations, male and female serum lipids, male and female age, male and female BMI, year of serum sample collection, and infertility diagnosis (female, male, unknown); P-value for trend (P-trend) was calculated as the median ln-transformed PBDE concentration of each quartile; RR: Relative risk; CI: Confidence interval; * Quartile is statistically different ($p<0.05$) from Q1 (reference)

Figure III.5. Relative risk (95% C) of clinical IVF outcomes by quartile of serum OH-BDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.



Cluster weighted generalized estimating equations adjusted for female serum OH-BDE concentration, paternal and maternal lipids, paternal and maternal age, paternal BMI, year of serum sample collection, and infertility diagnosis (female, male, unknown); The p-value for trend (P-trend) was calculated as the median ln-transformed serum OH-BDE concentration of each quartile; RR: Relative risk; CI: Confidence interval; * Quartile is statistically different ($p<0.05$) from Q1 (reference)

Chapter III Appendix

Table III.A.1. Demographic characteristics for 189 men from the EARTH cohort.

Characteristics	Median or n	(IQR or %)
Age (years)	36	(33, 40)
Race/ ethnicity		
Black/Asian/Other	27	(14.3)
White	162	(85.7)
Body mass index (kg/m^2)	27	(24, 29)
Ever smoker	59	(32)
Education		
High school/some college	13	(7)
College graduate	61	(32)
Graduate degree	115	(61)

IQR: Interquartile range

Table III.A.2. Regression coefficients (95% CI) of fertilization rate by quartile of serum PBDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.

	Fertilization Rate					
	Unadjusted Models			Adjusted Models [a]		
BDE	B	(95% CI)	p-value	B	(95% CI)	p-value
BDE47						
Q1	REF	REF	REF	REF	REF	REF
Q2	-0.28	(-0.65, 0.08)	0.13	-0.39	(-0.78, 0.004)	0.05
Q3	-0.07	(-0.45, 0.32)	0.73	-0.24	(-0.67, 0.19)	0.27
Q4	-0.27	(-0.65, 0.11)	0.16	-0.41	(-0.86, 0.04)	0.07
p-trend		0.28			0.16	
BDE99						
Q1	REF	REF	REF	REF	REF	REF
Q2	-0.34	(-0.72, 0.04)	0.08	-0.37	(-0.76, 0.01)	0.06
Q3	-0.27	(-0.65, 0.12)	0.17	-0.34	(-0.73, 0.05)	0.08
Q4	-0.37	(-0.75, 0.02	0.06	-0.36	(-0.76, 0.04)	0.08
p-trend		0.11			0.12	
BDE100						
Q1	REF	REF	REF	REF	REF	REF
Q2	-0.18	(-0.55, 0.19)	0.34	-0.32	(-0.69, 0.06)	0.10
Q3	0.21	(-0.19, 0.60)	0.30	0.01	(-0.39, 0.42)	0.95
Q4	-0.16	(-0.52, 0.21)	0.40	-0.31	(-0.69, 0.07)	0.11
p-trend		0.63			0.21	
BDE153						
Q1	REF	REF	REF	REF	REF	REF
Q2	0.02	(-0.36, 0.40)	0.92	0.08	(-0.30, 0.47)	0.67
Q3	-0.36	(-0.74, 0.02)	0.06	-0.35	(-0.74, 0.04)	0.08
Q4	0.03	(-0.35, 0.40)	0.89	0.04	(-0.34, 0.41)	0.85
p-trend		0.83			0.85	
BDE154						
Q1	REF	REF	REF	REF	REF	REF
Q2	0.34	(-0.03, 0.71)	0.07	0.35	(-0.03, 0.74)	0.07
Q3	0.15	(-0.22, 0.52)	0.42	0.22	(-0.18, 0.62)	0.27
Q4	0.03	(-0.34, 0.41)	0.87	0.09	(-0.33, 0.51)	0.68
p-trend		0.87			0.61	
BDE Sum						
Q1	REF	REF	REF	REF	REF	REF
Q2	0.05	(-0.33, 0.43)	0.79	0.05	(-0.34, 0.44)	0.79
Q3	0.25	(-0.14, 0.64)	0.21	0.23	(-0.17, 0.64)	0.26
Q4	0.05	(-0.31, 0.42)	0.78	0.08	(-0.30, 0.46)	0.69
p-trend		0.65			0.72	

[a] Models adjusted for adjusted for female serum PBDE concentrations, male and female serum lipids, male and female age, male and female BMI, year of serum sample collection, and infertility diagnosis (female, male, unknown); P-trend was calculated as the median ln-transformed serum PBDE concentration of each quartile; CI: Confidence interval; REF: Reference quartile

Table III.A.3. Regression coefficients (95% C) of fertilization rate by quartile of serum OH-BDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.

	Fertilization Rate					
	Unadjusted Models			Adjusted Models [a]		
OH-BDE	B	(95% CI)	p-value	B	(95% CI)	p-value
3&5-OH-BDE47						
Q1	REF	REF	REF	REF	REF	REF
Q2	0.10	(-0.27, 0.47)	0.58	0.07	(-0.31, 0.45)	0.71
Q3	0.33	(-0.06, 0.47)	0.10	0.32	(-0.08, 0.73)	0.11
Q4	-0.003	(-0.37, 0.37)	0.99	0.03	(-0.36, 0.42)	0.90
p-trend		0.99			0.85	
6-OH-BDE47						
Q1	REF	REF	REF	REF	REF	REF
Q2	-0.29	(-0.67, 0.08)	0.13	-0.25	(-0.64, 0.13)	0.19
Q3	-0.04	(-0.43, 0.36)	0.85	-0.08	(-0.48, 0.33)	0.71
Q4	-0.06	(-0.42, 0.31)	0.76	-0.004	(-0.39, 0.38)	0.98
p-trend		0.69			0.54	
4-OH-BDE49						
Q1	REF	REF	REF	REF	REF	REF
Q2	-0.23	(-0.61, 0.15)	0.23	-0.15	(-0.55, 0.25)	0.47
Q3	0.02	(-0.38, 0.41)	0.93	0.11	(-0.31, 0.53)	0.60
Q4	-0.05	(-0.43, 0.32)	0.77	0.07	(-0.32, 0.47)	0.71
p-trend		0.95			0.47	
OH-BDE Sum						
Q1	REF	REF	REF	REF	REF	REF
Q2	0.05	(-0.33, 0.43)	0.79	0.10	(-0.29, 0.49)	0.61
Q3	0.25	(-0.14, 0.64)	0.21	0.32	(-0.10, 0.74)	0.13
Q4	0.05	(-0.31, 0.42)	0.78	0.20	(-0.20, 0.60)	0.32
p-trend		0.65			0.28	

[a] Models adjusted for adjusted for female serum OH-BDE concentrations, male and female serum lipids, male and female age, male and female BMI, year of serum sample collection, and infertility diagnosis (female, male, unknown); The p-value for trend (P-trend) was calculated as the median ln-transformed serum OH-BDE concentration of each quartile; CI: Confidence interval; REF: Reference quartile.

Table III.A.4. Unadjusted relative risk (95% C) of clinical IVF outcomes by quartile of serum PBDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.

	Clinical IVF Outcomes								
	Implantation			Clinical			Live Birth		
BDE	RR	(95% CI)	p-value	RR	(95% CI)	p-value	RR	(95% CI)	p-value
BDE47									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.94	(0.70, 1.27)	0.70	0.81	(0.58, 1.13)	0.22	0.88	(0.57, 1.34)	0.54
Q3	0.96	(0.73, 1.25)	0.74	0.88	(0.65, 1.17)	0.37	0.82	(0.56, 1.21)	0.32
Q4	1.05	0.80, 1.36)	0.74	0.88	(0.64, 1.21)	0.43	0.99	(0.67, 1.46)	0.95
p-trend		0.69			0.54			0.97	
BDE99									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	**0.60**	(0.44, 0.82)	0.001	**0.60**	(0.42, 0.85)	0.004	**0.63**	(0.43, 0.93)	0.02
Q3	0.87	(0.67, 1.12)	0.27	0.77	(0.58, 1.02)	0.07	0.72	(0.48, 1.08)	0.12
Q4	0.86	(0.66, 1.11)	0.24	0.77	(0.56, 1.04)	0.09	0.84	(0.57, 1.24)	0.38
p-trend		0.79			0.31			0.67	
BDE100									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	**0.68**	(0.50, 0.92)	0.01	**0.65**	(0.47, 0.92)	0.02	0.66	(0.43, 1.03)	0.06
Q3	0.77	(0.59, 1.01)	0.06	0.78	(0.57, 1.05)	0.11	0.98	(0.67, 1.45)	0.93
Q4	0.93	(0.72, 1.19)	0.55	0.84	(0.62, 1.14)	0.28	0.99	(0.67, 1.46)	0.97
p-trend		0.90			0.64			0.57	
BDE153									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.95	(0.72, 1.25)	0.71	0.91	(0.66, 1.25)	0.56	0.88	(0.60, 1.31)	0.54
Q3	0.74	(0.54, 1.03)	0.07	0.75	(0.59, 1.05)	0.09	0.69	(0.45, 1.06)	0.09
Q4	1.06	(0.82, 1.38)	0.65	0.87	(0.64, 1.19)	0.38	0.91	(0.62, 1.35)	0.65
p-trend		0.81			0.31			0.56	
BDE154									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	1.36	(1.00, 1.84)	0.05	1.26	(0.88, 1.80)	0.20	1.29	(0.85, 1.94)	0.23
Q3	1.14	(0.83, 1.56)	0.42	1.08	(0.76, 1.55)	0.66	0.99	(0.64, 1.54)	0.97
Q4	1.16	(0.86, 1.57)	0.34	1.06	(0.74, 1.51)	0.76	1.01	(0.65, 1.57)	0.97
p-trend		0.49			0.89			0.82	
BDE Sum									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.96	(0.72, 1.29)	0.80	0.95	(0.69, 1.32)	0.78	1.07	(0.71, 1.59)	0.76
Q3	0.92	(0.68, 1.25)	0.60	0.88	(0.63, 1.23)	0.46	0.93	(0.60, 1.43)	0.75
Q4	1.09	(0.82, 1.44)	0.56	0.93	(0.66, 1.32)	0.69	1.02	(0.66, 1.58)	0.92
p-trend		0.55			0.64			0.95	

Cluster weighted generalized estimating equations; The p-value for trend (P-trend) was calculated as the median ln-transformed serum PBDE concentration of each quartile; RR: Relative risk; CI: Confidence interval; REF: Reference quartile; p-values <0.05 are in bold.

Table III.A.5. Unadjusted relative risk (95% C) of clinical IVF outcomes by quartile of serum OH-BDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.

	Clinical IVF Outcomes								
	Implantation			Clinical			Live Birth		
OH-BDE	RR	(95% CI)	p-value	RR	(95% CI)	p-value	RR	(95% CI)	p-value
3&5-OH-BDE47	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q1	0.76	(0.55, 1.03)	0.08	0.81	(0.57, 1.13)	0.22	1.04	(0.68, 1.59)	0.87
Q2	1.01	(0.77, 1.31)	0.96	0.94	(0.69, 1.27)	0.67	1.14	(0.75, 1.73)	0.54
Q3	0.95	(0.73, 1.23)	0.68	0.92	(0.66, 1.27)	0.60	1.12	(0.73, 1.72)	0.60
Q4									
p-trend		0.86			0.82			0.55	
6-OH-BDE47	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q1	0.99	(0.73, 1.34)	0.94	1.01	(0.72, 1.42)	0.96	0.96	(0.62, 1.47)	0.83
Q2	1.01	(0.75, 1.36)	0.96	1.02	(0.72, 1.46)	0.90	0.99	(0.65, 1.52)	0.98
Q3	1.14	(0.86, 1.52)	0.36	1.11	(0.79, 1.54)	0.56	1.18	(0.79, 1.74)	0.42
Q4									
p-trend		0.26			0.49			0.30	
4-OH-BDE49	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q1	1.13	(0.83, 1.54)	0.43	1.15	(0.82, 1.61)	0.43	1.16	(0.75, 1.78)	0.51
Q2	1.17	(0.88, 1.56)	0.28	1.10	(0.80, 1.53)	0.55	1.08	(0.71, 1.66)	0.72
Q3	1.19	(0.88, 1.60)	0.25	1.09	(0.78, 1.52)	0.63	1.28	(0.85, 1.94)	0.24
Q4									
p-trend		0.25			0.70			0.28	
OH-BDE Sum	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q1	1.36	(0.99, 1.86)	0.06	1.28	(0.90, 1.82)	0.18	**1.78**	(1.12, 2.83)	0.02
Q2	1.33	(0.95, 1.85)	0.10	1.19	(0.81, 1.73)	0.38	1.34	(0.81, 2.21)	0.25
Q3	1.33	(0.96, 1.84)	0.10	1.27	(0.88, 1.83)	0.21	**0.03**	(1.12, 2.85)	0.01
Q4									
p-trend		0.15			0.32			0.07	

Cluster weighted generalized estimating equations; The p-value for trend (P-trend) was calculated as the median ln-transformed serum OH-BDE concentration of each quartile; RR: Relative risk; CI: Confidence interval; REF: Reference quartile; p-values <0.05 are in bold.

Table III.A.6. Relative risk (95% C) of clinical IVF outcomes by quartile of serum PBDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.

	Clinical IVF Outcomes								
	Implantation			Clinical			Live Birth		
BDE	RR	(95% CI)	p-value	RR	(95% CI)	p-value	RR	(95% CI)	p-value
BDE47									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.86	(0.62, 1.20)	0.32	0.75	(0.52, 1.09)	0.14	0.80	(0.49, 1.29)	0.35
Q3	0.87	(0.63, 1.21)	0.39	0.81	(0.57, 1.17)	0.26	0.76	(0.48, 1.20)	0.24
Q4	0.89	(0.65, 1.23)	0.47	0.77	(0.53, 1.12)	0.18	0.84	(0.53, 1.33)	0.45
p-trend		0.65			0.32			0.62	
BDE99									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	**0.62**	(0.45, 0.84)	0.002	**0.61**	(0.42, 0.86)	0.01	**0.64**	(0.44, 0.91)	0.02
Q3	0.86	(0.66, 1.11)	0.25	0.75	(0.57, 1.00)	0.05	0.69	(0.47, 1.02)	0.06
Q4	0.86	(0.66, 1.11)	0.24	0.77	(0.57, 1.05)	0.10	0.83	(0.57, 1.20)	0.32
p-trend		0.63			0.24			0.52	
BDE100									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	**0.62**	(0.45, 0.86)	0.004	**0.59**	(0.41, 0.84)	0.004	**0.56**	(0.37, 0.87)	0.01
Q3	0.77	(0.58, 1.01)	0.06	0.76	(0.55, 1.04)	0.09	0.93	(0.61, 1.40)	0.71
Q4	0.85	(0.65, 1.10)	0.20	0.76	(0.55, 1.03)	0.08	0.86	(0.59, 1.26)	0.43
p-trend		0.73			0.34			0.97	
BDE153									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.92	(0.69, 1.23)	0.57	0.88	(0.62, 1.24)	0.47	0.82	(0.54,	0.28
Q3	**0.63**	(0.46, 0.86)	0.004	**0.66**	(0.47, 0.92)	0.02	**0.62**	1.25)	0.03
Q4	0.98	(0.76, 1.25)	0.85	0.81	(0.61, 1.09)	0.17	0.86	(0.40, 0.96) (0.59, 1.26)	0.45
p-trend		0.74			0.14			0.46	
BDE154									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	**1.37**	(1.01, 1.82)	0.04	1.27	(0.89, 1.82)	0.19	1.33	(0.88, 2.02)	0.17
Q3	1.15	(0.84, 1.59)	0.39	1.11	(0.77, 1.61)	0.58	1.09	(0.70, 1.70)	0.70
Q4	1.29	(0.93, 1.78)	0.13	1.18	(0.80, 1.74)	0.41	1.20	(0.76, 1.90)	0.43
p-trend		0.21			0.50			0.53	
BDE Sum									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.86	(0.64, 1.18)	0.37	0.85	(0.59, 1.22)	0.38	1.00	(0.65, 1.56)	0.90
Q3	0.86	(0.63, 1.17)	0.34	0.82	(0.57, 1.18)	0.29	0.92	(0.58, 1.46)	0.61
Q4	0.94	(0.69, 1.27)	0.68	0.80	(0.55, 1.17)	0.25	0.92	(0.58, 1.48)	0.67
p-trend		0.84			0.29			0.66	

Cluster weighted generalized estimating equations adjusted for female serum PBDE concentrations, male and female serum lipids, male and female age, and male and female BMI, year of serum sample collection, and infertility diagnosis (female, male, unknown); The p-value for trend (P-trend) was

calculated as the median ln-transformed serum PBDE concentration of each quartile; RR: Relative risk; CI: Confidence interval; REF: Reference quartile; p-values <0.05 are in bold

Table III.A.7. Relative risk (95% C) of clinical IVF outcomes by quartile of serum OH-BDE concentrations among 189 men (285 IVF cycles) from the EARTH cohort.

	Clinical IVF Outcomes								
	Implantation			Clinical			Live Birth		
OH-BDE	RR	(95% CI)	p-value	RR	(95% CI)	p-value	RR	(95% CI)	p-value
3&5-OH-BDE47									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.77	(0.57, 1.05)	0.10	0.82	(0.58, 1.15)	0.25	1.03	(0.67, 1.59)	0.89
Q3	1.04	(0.79, 1.39)	0.77	0.97	(0.69, 1.36)	0.86	1.21	(0.78, 1.86)	0.39
Q4	0.96	(0.73, 1.26)	0.77	0.92	(0.66, 1.29)	0.65	1.21	(0.78, 1.87)	0.40
p-trend		0.92			0.81			0.36	
6-OH-BDE47									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	0.98	(0.72, 1.34)	0.91	1.02	(0.71, 1.46)	0.91	0.99	(0.64, 1.53)	0.96
Q3	0.95	(0.70, 1.28)	0.73	0.96	(0.67, 1.37)	0.81	0.90	(0.60, 1.34)	0.59
Q4	1.08	(0.81, 1.45)	0.60	1.07	(0.76, 1.52	0.68	1.16	(0.78, 1.75)	0.46
p-trend		0.49			0.66			0.38	
4-OH-BDE49									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	1.02	(0.73, 1.42)	0.92	1.03	(0.72, 1.48)	0.86	0.94	(0.60, 1.48)	0.80
Q3	1.07	(0.78, 1.46)	0.68	1.04	(0.73, 1.47)	0.84	0.99	(0.63, 1.57)	0.96
Q4	1.07	(0.79, 1.47)	0.65	0.98	(0.69, 1.40)	0.93	1.08	(0.70, 1.66)	0.73
p-trend		0.61			0.90			0.63	
OH-BDE Sum									
Q1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Q2	1.41	(1.00, 2.00)	0.06	1.35	(0.91, 2.00)	0.13	**2.17**	(1.34, 3.53)	0.001
Q3	1.34	(0.93, 1.93)	0.12	1.20	(0.80, 1.82)	0.38	1.53	(0.91, 2.58)	0.11
Q4	1.34	(0.93, 1.92)	0.12	1.31	(0.87, 1.97)	0.20	**2.12**	(1.29, 3.49)	0.003
p-trend		0.23			0.34			**0.03**	

Cluster weighted generalized estimating equations adjusted for female serum OH-BDE concentrations, male and female serum lipids, male and female age, male and female BMI, year of serum sample collection, and infertility diagnosis (female, male, unknown); The p-value fore trend (P-trend)was calculated as the median ln-transformed serum OH-BDE concentration of each quartile; RR: Relative risk; CI: Confidence interval; REF: Reference quartile; p-values <0.05 are in bold.

Figure III.A.1. Distributions of serum OH-BDEs (ng/g lipid) among 189 couples from the EARTH study.

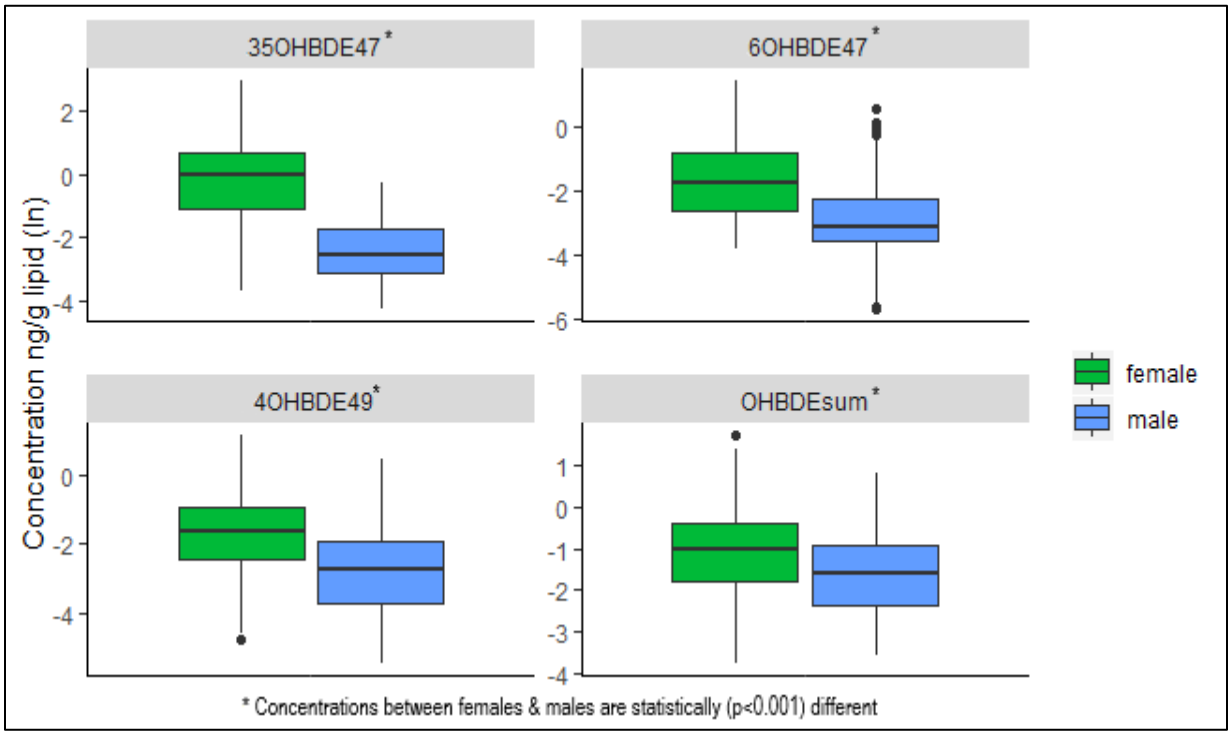
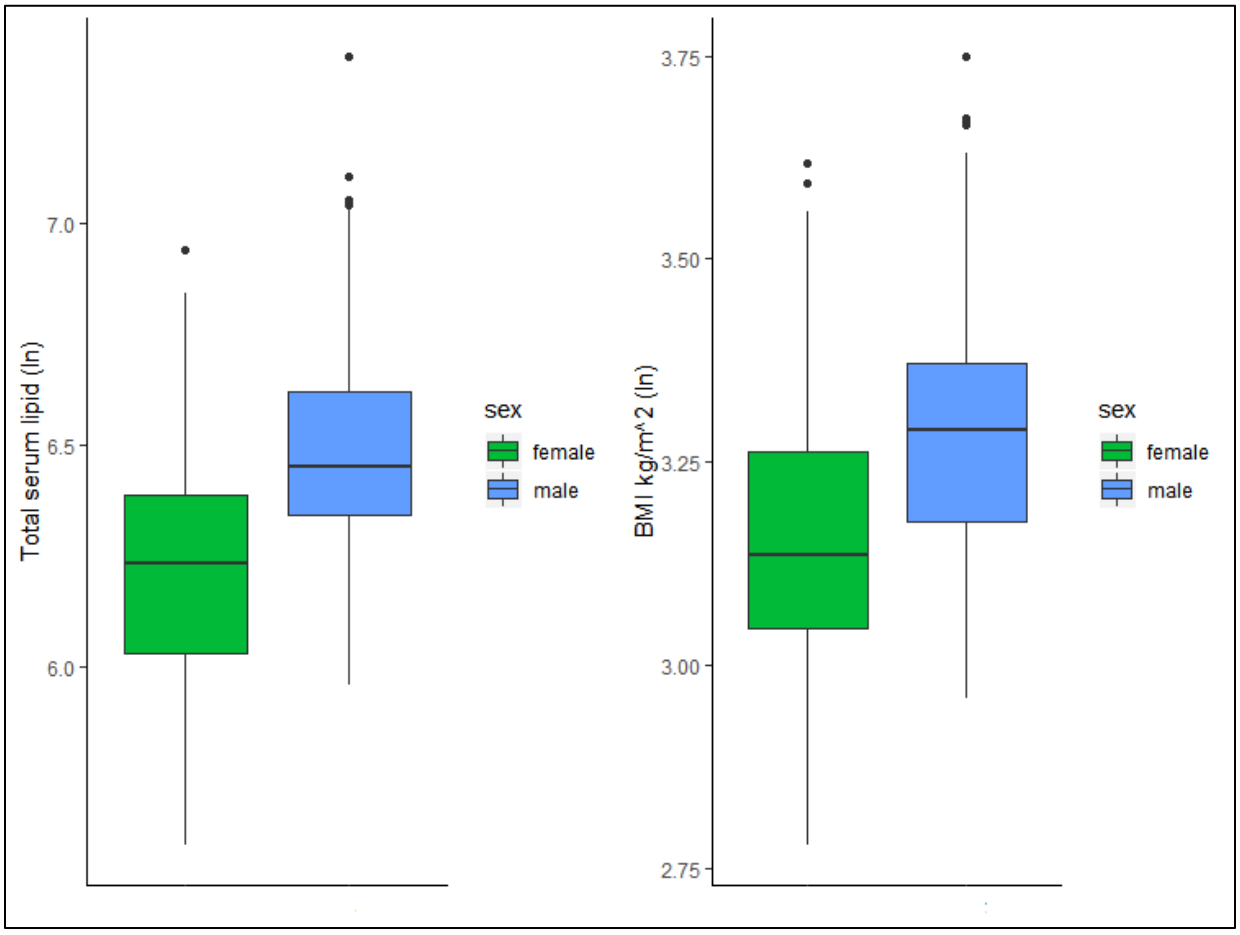


Figure III.A.2. Distributions of total serum lipids and BMI (kg/m^2) among 189 couples from the EARTH study.



CHAPTER IV

AIM 3 Part A: The association of Urinary Organophosphate Ester Metabolites and self-Reported Personal Care and Household Product Use Among Couples Seeking Fertility Treatment

Abstract

Background: Organophosphate esters (OPEs) are widely detected. They are used both as flame retardants as well as plasticizers.

Methods: 230 women and 229 men, a subset of a larger cohort, were recruited from Massachusetts General Hospital fertility clinic between 2005 and 2015. At each visit, participants completed a questionnaire of personal care product (PCP) and household product (HP) use. Metabolites [bis(1,3-dichloro-2-propyl) phosphate, diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate and bis(1-chloro-2-propyl) phosphate] were measured in urine (1-5 samples; n=638 women, n=335 men). Associations were assessed using generalized mixed models, adjusted for specific gravity, age, BMI, smoking, education and season.

Results: In women, moisturizer (60%), nail polish remover (77%) and nail polish (134%) use were associated ($p<0.05$) with an increase in DPHP concentrations, while ip-PPP concentrations increased 21-27% with conditioner, cosmetics, deodorant and hair product use. Mouthwash and vinyl glove use were associated with a respective 31 and 92% increase in DPHP among men.

Conclusions: Our exploratory analysis suggests OPEs used as a plasticizer in consumer products, and nail polish use contributes to internal DPHP exposure. Further research is needed to understand how OPEs are used in these products and how it relates to exposure.

Introduction

Organophosphate esters (OPEs) have been used as flame retardants (FR) for over 150 years [1,2]. The use of OPEs has grown drastically since the phase out of polybrominated diphenyl ethers (PBDEs) in the past decade due to concerns regarding their persistence and toxicity [3–5]. As their prevalence rose, OPEs evolved into a high production volume chemical with US production projected to reach approximately 50,000 tons per year by 2020 for certain compounds [6]. OPEs include both chlorinated alkyl esters such as tris(2-chloroisopropyl) phosphate (TCIPP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP), and non-halogenated aryl phosphates such as triphenyl phosphate (TPHP) and isopropyl triphenyl phosphate (ITP) [7]. TPHP and ITP account for 60% of Firemaster® 550, a widely used commercial flame retardant mixture that replaced PBDEs in furniture foams and baby products, yet are also used as a plasticizer in paints, lacquers, and varnishes [8–11].

Considered 'additive' compounds, OPEs are physically added or 'mixed' with materials during manufacturing, rather than being chemically bound [10,12]. The main route of exposure of OPEs was thought to be dust ingestion as a result of the weak bonds allowing for volatilization and settlement into dust of indoor environments [13,14]. However, recent studies using air samplers, hand wipes and silicone wrist bands have shown that inhalation and dermal exposure may also be pathways of exposure [6,11,15]. Despite the short biological half-lives ranging from a few hours to days, metabolites of OPEs have been detected in nearly 100% of urine samples among women, men, and children in the US and Europe [14,16–19]. Despite being rapidly metabolized once in the body, high detection of OPEs suggests exposure is continuous and widespread.

Research on the health effects of OPEs is limited, although prior studies have shown adverse immunologic and neurologic outcomes, as well as associations with the disruption of endocrine, reproductive, and developmental systems [20–24]. As of 2011, TDCIPP and tris(2-chloroethyl) phosphate (TCEP) are listed as a known carcinogens by the state of California [25].

These compounds have been highly detected in environmental samples, and primary sources are thought to be polyurethane foams found in furniture, baby products, and electronics [26–28] . Few studies to date have assessed the prevalence of OPEs in other consumer products and personal care products (PCPs) where they may be utilized as a plasticizer. A widely used OPE, TPHP is commonly listed as an ingredient in nail polishes. Several studies have also detected TPHP in products where it was not listed as an ingredient [29–31]. A small study found urine concentrations of diphenyl phosphate (DPHP), a metabolite of TPHP, to increase 7-fold after nail polish application [30]. Our present work expands upon this preliminary evidence to characterize the relationship between self-reported PCP and household product (HP) use within 24 hours of a urine sample measuring the concentration of five OPE metabolites: bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), DPHP, isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) among couples attending a fertility clinic.

Materials and Methods

Participant Recruitment

Couples from this analysis are a subset from the EARTH study, an existing prospective cohort assessing the impact of environmental agents on reproductive

health. Recruitment and participation have been previously described [23,32]. Briefly, women (18-46 yrs.) and men (18-55 yrs.) were recruited from Massachusetts General Hospital (MGH) Fertility Center between 2005-2015. Among couples approached for the EARTH study, approximately 60% of women and 50% of men agreed to participate. Women were included in this analysis if they provided at least one urine sample for OPE measurement and completed the PCP and HP questionnaire during an *in vitro* fertilization cycle, while men must have provided at least one urine sample for OPE measurement, completed the questionnaire, and have a woman partner also in the study [33]. Men were excluded only if they had a prior vasectomy. Informed consent was given by each participant and Institutional Review Board approval was received by all institutions.

Personal Care Product (PCP) and Household Product (HP) Questionnaires

At the time of enrollment, couples completed questionnaires capturing demographic, health history, and lifestyle factors. At the beginning of each subsequent visit, women and men completed a questionnaire on PCP (n=20 products) and HP (n=14 products) use within the last 24 hrs. Consumer products with n<5 participants reporting use in the last 24 hrs. was excluded from the analysis.

Urine Collection and Phosphorous-Containing Flame Retardant Analysis

One urine sample (up to five samples per participant) were collected in sterile polypropylene cups from couples at each visit. After collection, specific gravity (SG) was measured for each sample using a Protometer handheld 100B refractometer (National Instrument Company, Inc., Austin, TX). Samples were then separated into

aliquots and frozen (-80°) prior to overnight shipment on dry ice to H.M. Stapleton's laboratory at Duke University (Durham, NC) for analysis.

Extraction and analysis of OPE metabolites BCIPP, BDCIPP, DPHP, ip-PPP, and tb-PPP have been established and previously described [34]. Briefly, samples were thawed and separated into glass tubes in 5 mL aliquots and spiked with internal standards (d_{10}-BDCIPP = 80 ng, d_{10}-DPHP = 60 ng). Samples were then acidified to pH <6.5 with formic acid and diluted 1:1 with water. Samples were concentrated and cleaned using solid phase extraction (SPE) before nitrogen stream drying and then spiked with recovery standard ($^{13}C_2$-DPHP = 81.5 ng). Extracts were analyzed using negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS). Data were acquired using optimal parameters under multiple reaction conditions. Internal standard used for BCIPP and BDCIPP was d_{10}-BDCIPP, while DPHP, ip-DPHP, and tb-PPP were quantified using d_{10}-DPHP. Urinary SG ranged from 1.002-1.100 (geometric mean (GM)=1.104) for women and 1.011-1.038 (GM=1.017) for men.

Procedures for quality control and assurance for LC-MS/MS have been previously reported [23]. Samples were analyzed in 10 separate batches including five blanks (5 mL Mili-Q water) to establish a distinct method detection limit (MDL) for each batch. Laboratory blanks were multiplied three times the standard deviation to establish MDLs which ranged from: 0.07-0.17 pg/mL for BCIPP, 0.02-0.11 pg/mL for BDCIPP, 0.09-0.18 pg/mL for DPHP, 0.06-0.12 pg/mL for ip-PPP, and 0.04-0.15 pg/mL for tb-PPP. A standard reference material (SRM) was established using pooled samples from prior studies and precision was evaluated with duplicates of two-subsamples.

Statistical Analysis

Distributions of OPEs have been previously reported for participants [23,35]. Due to the low frequency of detectable concentrations, BCIPP was henceforth excluded in this analysis. Metabolite concentrations below MDL were imputed as MDL/√2. An aggregate variable (∑OPE) was imputed by summing the molar urinary concentrations for metabolites BDCIPP, DPHP, and ip-PPP. Spearman correlation coefficients were calculated for each metabolite among 229 couples. Metabolites BDCIPP, DPHP, ip-PPP, and ∑OPE presented as right-skewed and were therefore transformed by the natural logarithm for further statistical modeling.

Questionnaire responses to PCP and HP use within the last 24 hours was evaluated as binary ('yes' or 'no') where those who responded 'don't know' (n<9 per consumer product) were re-coded as a 'no' response. OPE metabolites were evaluated as continuous variables except for tb-PPP, which had low detection rates (13.32% for women and 11.34% for men) and was modeled as detect/non-detect (data not shown). Covariates for modeling were selected a priori and through bivariate testing (data not shown) [32,36,37]. Final models were adjusted for SG, age, BMI, race (other/Caucasian) smoking (never/ever), education (high school, some college/college, or graduate/graduate degree), and season (winter/spring/summer/fall). Models that included year of collection had decreased goodness of fit and are not presented. Missing covariates were imputed with the median for continuous variables (age=34 for women, n= 16 and BMI=26.84 for men, n=3) and the category with the highest frequency (education=graduate degree, n=61 women and n=38 men). Multivariable generalized mixed models were used to evaluate associations with repeated PCP and

HP use (exposure) and OPE metabolite concentrations (outcome) using a normal distribution with identity link (Supplemental Tables IV.1-4). Regression coefficients and 95% confidence intervals (CI) were transformed to reflect the adjusted percent change in urinary OPE metabolite concentrations with reported use of each PCP and HP within 24 hrs. of urine sample (Supplemental Tables IV.5-8). Heat maps were then generated to graphically display the adjusted percent change (Figures IV.1-4; statistical significance is indicated by an asterisks on maps). A sensitivity analysis dividing observations into five year increments (2005-2009 and 2010-2015) was conducted to investigate possible changes in consumer product formulations by time period (data not shown). All statistical analyses were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Our subset from the EARTH cohort consisted of 230 women and 229 men contributing one to five urine samples per participant (n=638 women and n=335 men). Demographic characteristics of these women and men have been previously reported [23,35]. Briefly, this sample consisted primarily of Caucasian (87%), non-smoking (75%), and highly educated (57% hold graduate degree) women with an average age of 35 [23]. Men were slightly older than women (Mean=36.78 yrs.), yet also predominantly White (89%), non-smokers (70%) with 82% holding a college degree or higher [35].

Self-reported PCP use for women and men within 24 hrs. of urine sample collection are depicted in Table IV.1. The most commonly used PCPs by women were deodorant (n=378), shampoo (n=367), toothpaste (n=364), conditioner/cream rinse (n=323), and bar soap (n=321). Suntan/sun block lotion (n=49), nail polish remover

(n=23), and nail polish (n=21) had the lowest reported use. Similarly, men also frequently reported using deodorant (n=211), shampoo (n=204), toothpaste (n=190), and bar soap (n=177), but also shaving cream (n=87). Less frequently reported PCPs among men included aftershave, other hair products, and suntan/sunblock lotion (<10%). Reported HP use was less frequent among women and men compared to PCP (Table IV.2). However, both women and men reported use of laundry detergent, hand dishwashing liquid and cleaners. For women, vinyl gloves, furniture polish, and vinyl boots were less frequently used (<6%), while vinyl gloves, fabric softener, and paint/solvents were the least reported HP for men (<5%).

Metabolites BDCIPP, DPHP, and ip-PPP were highly detected among both women (85%, 90%, and 75%) and men (85%, 86%, and 67%) [23,35]. Metabolite concentrations were higher among women compared to men for BDCIPP and DPHP, similar for ip-PPP, while tb-PPP concentrations were higher in men. Metabolite concentration correlations among couples (n=229) were weak for all metabolites ($0.20<r<0.31$, $p\leq0.01$), with the exception of tb-PPP ($r=0.70$, $p=0.04$), that was detected at low rates (<15% above MDL) for both women and men (Table IV.3). Concentrations of DPHP for men were also weakly correlated to ip-PPP concentrations in women ($r=0.14$, $p=0.04$).

Adjusted percent change in OPE metabolite concentrations with self-reported PCP use for women can be found in Figure IV.1. Use of nail polish was associated with a 134% increase (95% CI: 62, 235; $p<0.0001$) in DPHP concentrations as well as nail polish remover (77%; 95% CI: 21, 159; $p=0.0004$). Increased concentrations of DPHP were associated with reported use of face moisturizer (60%; 95% CI: 15, 123; $p=0.01$).

Deodorant use was also associated with a 28% increase in DPHP (95% CI: 5, 57; p=0.02) and a 26% increase in ip-PPP (95% CI: 1, 55; p=0.04) concentrations. We identified significant relationships with the use of colored cosmetics with DPHP and ip-PPP concentrations (27%, 95% CI: 7, 49; p=0.01 and 26%, 95% CI:1, 55; p=0.01 respectively). Reported use of hair products including mousse, hair bleach, relaxer, and perm straightener was associated with a 27% increase (95% CI: 2, 58; p=0.04) in ip-PPP concentrations while toothpaste was associated with a non-significant increase of 80% (95% CI: -35, 395; p=0.25). Yet for men, only mouthwash was significantly associated with a 31% increase in DPHP (95% CI: 3, 63; p=0.03) and total OPE (95% CI: 8, 62; p=0.01) concentrations (Figure IV.2).

Vinyl glove use was associated with a 32% increase in BDCIPP and ip-PPP concentrations among women (95% CI: -20, 120; p=0.27 and 95% CI: -11, 97; p=0.17), while concentrations of DPHP were related to a 27% decrease (95% CI: -45, -3; p=0.03) with reported hand dishwashing liquid use (Figure IV.3). Vinyl glove use in men had the highest associated increase in BDCIPP (95% CI: -12, 314; p=0.10), DPHP (95% CI: 9, 232; p=0.02), and total OPE (95% CI: 3, 175; p=0.04) concentrations (92%, 92%, and 68%, respectively) (Figure IV.4). While not statistically significant, concentrations of BDCIPP were associated with an 85% increase with the use of paints/solvents (95% CI: -73, 169; p=0.77) while fabric softener use was related to an 57% increase in BDCIPP (95% CI: -74, 25; p=0.16) and 63% increase in DPHP (95% CI: -65, 12; p=11) concentrations.

In our sensitivity analysis, nail polish use was associated with a 306% increase in urinary DPHP concentrations (95% CI: 129, 610; p<0.0001) among women with

samples collected between 2010-2015. Metabolite concentrations of ip-PPP in women increased by 40% in association with deodorant use during 2010-2015 (95% CI: 5, 88; p=0.02). All significant associations with OPEs and PCP and HP highlighted in our primary analysis remained the same or similar when only using observations collected during 2010 or later. However, these associations disappeared when only using observations between 2005-2009 (data not shown). No other significant associations were identified during this secondary analysis.

Discussion

We identified several associations between PCP use and DPHP concentrations, specifically with the use of nail polish, nail polish remover, face moisturizer, deodorant, and cosmetics in women. Concentrations of ip-PPP were also associated with increased reported use of deodorant, cosmetics, and hair products. We did not observe similar relationships with PCP use in men, only finding associations with DPHP and total OPE with mouthwash. Overall, there were few significant associations identified with HP use. Reported use of vinyl gloves was associated with elevated concentrations of all metabolites for both men and women. Although most product use was associated with an increase in OPE metabolite concentration, dishwashing liquid use was significantly associated with a decrease in DPHP concentrations among women.

We found OPE concentrations to be slightly higher in women compared to men for BDCIPP and DPHP in this study. The National Health and Nutrition Examination Survey (NHANES) reported similar results with slightly higher concentrations of DPHP in women (GM=0.92 μg/L) compared to men (GM=0.78 μg/L), yet concentrations of BDCIPP were slightly higher for men (GM=0.91 μg/L) compared to women (GM=0.80

μg/L) [3]. Concentrations of DPHP for men and women from NHANES were similar to our sample (women GM=0.91, men GM=0.75 μg/L), though BDCIPP metabolite concentrations were slightly higher for women (GM=0.91 μg/L) and lower for men (GM=0.62 μg/L). A small study (n=53) in North Carolina found a slightly larger sex disproportion of DPHP with women having approximately a 2-fold higher urinary concentration (10^{β} =1.84) compared to men (10^{β} =0.98), although BDCIPP concentrations were comparable [12]. Similar to our findings, phthalate metabolites have also been found at higher concentrations in women compared to men, and phthalates are also used as plasticizers in various PCPs and HPs [38,39].

Along with the differences in OPE concentrations by sex, the lack of similar relationships between self-reported PCP use and OPE metabolite concentrations could possibly be a result of the episodic use patterns of PCPs, lifestyle, as well as different formulations of products targeted to each sex [3,33]. A US survey of 2,300 adults found the average women uses 12 products consisting of approximately 168 unique ingredients per day while men use an average of 6, exposing them to 85 unique chemicals in a single day [40]. Higher usage of both PCP and HP among women has also been reported in studies from the Netherlands, Switzerland, and South Korea [41–43].

Organophosphates have been largely associated with their use as FRs in polyurethane foam in furniture and cars, electronics, as well as components of the widely used FR mixture Firemaster ® 550 [28,44–46]. However, non-halogenated compounds like TPHP and ITP are also used as plasticizers [8]. Plasticizers are frequently used to increase the flexibility of plastics and in the production of vinyl in PCP and HP [47,48]. This coincides with the majority of our relationships identified with

increasing concentrations of TPHP and ITP metabolites (DPHP and ip-PPP, respectively) with reported use of PCP and HP. TPHP is commonly listed as an ingredient in nail polishes where it is likely used to increase the flexibility of the polish after its application. A small study from the California Environmental Protection Agency (Cal EPA) detected TPHP in five of 14 nail products tested [29]. Interestingly, when TPHP was detected, a common plasticizer, dibutyl phthalate (DBP) was not found. Thus, TPHP is possibly replacing DBP in nail products as a plasticizer. This potentially explains our strongest association of percent increase for DPHP concentrations (134%) with reported nail polish use which more than doubled (306%) when only using observations collected during 2010 or later. Our findings also overlap with a prior study of urinary DPHP concentrations and nail polish application that found a larger increase in urine concentrations (7-fold) compared to our results, which could be a result of more rigorous and timely urine collection in their study design [30]. We also detected a significant association with nail polish remover and elevated DPHP concentrations (77%). This result was unexpected as acetone, ethylene glycol, and gamma butyrolactone are the most common ingredients for nail polish remover [49]. While this could be a result of OPEs in the product, it is also possible that as nail polish is being removed, exposure via inhalation or dermal absorption is increased. Or, nail polish remover could be acting as a surrogate for nail polish as use of both products was correlated (p=0.001). Deodorant use among women was also correlated with nail polish use (p=0.02) and surrogacy possibly explains this unexpected result. In our secondary analysis we also observed a 40% increase in ip-PPP urine concentrations using observations between 2010-2015 (compared to a 20% increase using all observations)

for women who reported using deodorant. Nail polish and deodorant were the only products to have a substantial difference in OPE concentrations when exploring the year of collection. Along with TPHP replacing DBP as a plasticizer in nail polish, it is also possible that phthalates used in deodorants are also being replaced with OPES like TPHP or ITP [50]. These results further highlight the possibility of OPEs replacing phthalates as plasticizers around 2010 [31].

We also observed a significant decrease in DPHP concentrations with reported dishwashing liquid use. This is possibly due to reduced dermal absorption as a result of frequent hand washing which has been reported for several OPEs [51]. Although not significant, we also observed decreased OPE concentrations with reported conditioner and bar soap use in women and shampoo, toothpaste, and shaving cream use among men, which could also be a result of washing, rinsing, or bathing to decrease dermal absorption.

Weak correlations of metabolites among couples suggest that dust ingestion from OPEs in furniture foams from the home is not the sole exposure route/pathway. Organophosphate esters are characterized as semi volatile organic compounds and continuously divided between the gaseous and solid phases. Thus, exposure to OPEs is possibly from multiple routes/pathways in which they are ingested as well as absorbed through the skin from PCP and HP use [52]. These weak correlations could be a result of differences in PCP and HP use patterns between sexes, or varying metabolism rates of OPEs among women and men.

Although novel, our study was subject to several limitations. The PCP and HP questionnaire did not capture frequency, amount, or the last time of product use in

relation to urine sample collection. However, due to the exploratory nature of this study, additional adjustment for these factors may have saturated our model and biased our results towards the null. OPE exposure differences among couples could be a result of being in different environments throughout the day as OPEs have also been highly detected in cars and offices [7]. Despite being a comprehensive PCP and HP use questionnaire, our results may be susceptible multiple statistical comparisons. Nevertheless, our results coincide with the prior studies of DPHP and nail polish [30].

Several factors may have attenuated the effect estimates in our analysis. Due to the rapid metabolism of OPEs, resulting in half-lives of several hours, as well as the episodic nature of PCP and HP use, our effect sizes of our relationships may be underestimated [17,33]. However, studies from the same cohort found moderate temporal variability among OPE concentrations over a three month time period [17,23]. While our study consisted of approximately 230 couples, we had almost twice as many women's urine samples (n=638) compared to men's (n=335). A difference in repeated measurements could explain the sex differences we observed in our relationships with PCP and HP. OPE concentrations in women have also been found to be higher compared to men, consistent with ?our present findings [3]. Our study population was comprised primarily of White, non-smoking, highly educated couples who were subfertile, which could also have resulted in modest results as prior studies have found higher OPE concentrations associated with lower socioeconomic status (SES) and non-White populations [3,19]. Thus, our results may only be generalizable to similar populations, yet they identify the necessity to investigate these associations in diverse populations.

Our study also had several strengths. To the best of our knowledge, we are the most comprehensive study to date to assess the potential relationships of OPE metabolite concentrations with self-reported PCP and HP use. Our study design also allowed for increased precision in measurement of OPE metabolites due to multiple urine samples as well as multiple questionnaire responses per participant. The prospective study design also decreased the possibility of systematic error as the questionnaire referenced product use only 24 hrs. prior to urine sample collection. Also, as our sample consisted of couples, we were able to identify the lack of correlation between metabolites among couples likely residing in the same residence to exploit the possibility for alternate exposure routes and pathways besides dust from the home. Finally, the longevity of this well-established cohort spanning 10 years of sample collection allowed for the exploration of temporal associations correlating to formulation changes of PCP and HPs.

Conclusion

To the best of our knowledge, this is the first study to characterize the relationships of OPE metabolites and self-reported PCP and HP use. While metabolites BDCIPP, DPHP, and ip-PPP were highly detected among women and men, concentrations in women were slightly higher. Correlations of metabolites were weak among couples which is consistent with our different results among sexes. Similar to the only prior study of DPHP in urine and nail polish, we identified an association of increased DPHP concentrations in urine (134%) with reported nail polish use. This association nearly doubled (306% increase) when only using observations from 2010-2015. We also identified significant associations with reported use of other PCPs

including: nail polish remover, face moisturizer, colored cosmetics, and deodorant for women. Relationships with HP use were fewer, yet vinyl glove use in men was associated with a 92% increase in DPHP concentrations. These results suggest OPEs are not only used as FRs, but possibly as plasticizers in these products and also contribute to internal exposure. Furthermore, it is possible this replacement occurred during 2010 as highlighted by our differences in associations using observations between 2005-2009 compared to those between 2010-2015. Our results identify the necessity of more targeted studies to further investigate the prevalence of OPE compounds, especially non-halogenated aryl phosphates TPHP and ITP, in PCPs and HPs highlighted by our results.

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Table IV.1: Summary of reported personal care product use within 24 hours of OPE urine sample collection among Environment and Reproductive Health (EARTH) study participants between 2005-2015 (n=230 women; n=229 men).

Personal Care Product	Yes (n, %)	No (n, %)	Missing (n, %)
Women (n=638 samples)			
Deodorant	(378, 59.2)	(101, 15.8)	(159, 24.9)
Shampoo	(367, 57.5)	(112, 17.6)	(159, 24.9)
Toothpaste	(364, 57.1)	(3, 0.4)	(271, 42.5)
Conditioner / Cream Rinse	(323, 50.6)	(156, 24.5)	(159, 24.9)
Bar Soap	(321, 50.31)	(157, 24.6)	(160, 25.1)
Hand/Body Lotion	(298, 46.8)	(181, 28.4)	(159, 24.9)
Colored Cosmetics	(203, 31.8)	(276, 43.3)	(159, 24.9)
Face Moisturizer/ Lotion	(146, 22.9)	(37, 5.8)	(455, 71.3)
Hair Spray/ Hair Gel	(134, 21.0)	(341, 53.4)	(163, 25.5)
Liquid Soap/ Body Wash	(128, 20.1)	(55, 8.6)	(455, 71.3)
Mouthwash	(115, 18.0)	(349, 54.7)	(174, 27.3)
Cologne or Perfume	(114, 17.9)	(365, 57.2)	(159, 24.9)
Other Toiletries	(94, 14.7)	(54, 8.4)	(490, 76.8)
Other hair products	(92, 14.4)	(386, 60.5)	(160, 25.1)
Hand Sanitizer	(80, 12.5)	(286, 44.8)	(272, 42.6)
Shaving Cream	(76, 11.9)	(403, 63.2)	(159, 24.9)
Suntan / Sunblock Lotion	(49, 7.7)	(430, 67.4)	(159, 24.9)
Nail Polish Remover	(23, 3.6)	(159, 24.9)	(456, 71.5)
Nail Polish	(21, 3.3)	(457, 71.6)	(160, 25.1)
Men (n=335 samples)			
Deodorant	(211, 63.0)	(37, 11.0)	(87, 26.0)
Shampoo	(204, 60.9)	(44, 13.1)	(87, 26.0)
Toothpaste	(190, 56.7)	(4, 1.2)	(141, 42.1)
Bar Soap	(177, 52.8)	(71, 21.2)	(87, 26.0)
Shaving Cream	(87, 13.6)	(161, 43.8)	(87, 26.0)
Mouthwash	(79, 23.6)	(169, 50.4)	(87, 26.0)
Hair Spray/ Hair Gel	(74, 22.1)	(174,51.9)	(87, 26.0)
Hand/Body Lotion	(63, 18.8)	(184, 54.9)	(88, 26.3)
Liquid Soap/ Body Wash	(59, 17.6)	(48, 14.3)	(228, 68.1)
Hand Sanitizer	(54, 16.1)	(139, 41.5)	(142, 42.4)
Cologne or Perfume	(41, 12.2)	(206, 61.5)	(88, 26.3)
Conditioner / Cream Rinse	(40, 11.9)	(208, 62.1)	(87, 26.0)
Face Moisturizer/ Lotion	(18, 5.4)	(90, 26.9)	(227, 67.8)

Aftershave	(16, 4.8)	(232, 69.3	(87, 26.0)
Other hair products	(16, 4.8)	(230, 68.7)	(89, 26.6)
Other Toiletries	(14, 4.18)	(77, 23.0)	(244, 72.8)
Suntan / Sunblock Lotion	(7, 2.1)	(241, 71.9)	(87, 26.0)

Other hair products include: mousse, hair bleach, relaxer, perm straightener; Other toiletries include: wax, Vaseline, lip balm; Reported use of Personal care products n <5 were not listed

Table IV.2: Summary of reported household product use within 24 hours of OPE urine sample among EARTH participants between 2005-2015 (n=230 women; n=229 men).

Household Product	Yes (n, %)	No (n, %)	Missing (n, %)
Women (n=638)			
Laundry Detergent	(147, 23.1)	(332, 52.3)	(159, 24.9)
Hand Dishwashing Liquid	(123, 19.3)	(60, 9.4)	(455, 71.3)
Cleaners	(99, 15.5)	(380, 59.6)	(159, 24.9)
Fabric Softener	(63, 9.9)	(416, 65.2)	(159, 24.9)
Vinyl Gloves	(28, 4.4)	(451, 70.7)	(159, 24.9)
Furniture Polish	(9, 1.4)	(470, 73.7)	(159, 24.9)
Vinyl Boots	(8, 1.3)	(471, 73.8)	(159, 24.9)
Men (n=335)			
Hand Dishwashing Liquid	(59, 17.6)	(49, 14.6)	(227, 67.8)
Cleaners	(38, 11.3)	(210, 62.7)	(87, 26.0)
Laundry Detergent	(22, 6.6)	(226, 67.5)	(87, 26.0)
Vinyl Gloves	(9, 2.7)	(238, 71.0)	(88, 26.3)
Fabric Softener	(8, 2.4)	(240, 7.2)	(87, 26.0)
Paint/ Solvents	(5, 1.5)	(103, 30.7)	(227, 67.8)

Reported use of Household products n <5 were not listed

Table IV.3: Spearman correlation coefficients of urinary OPE metabolites among couples in the EARTH cohort (n=229 couples).

		Women OPE metabolites							
		BDCIPP		DPHP		ip-PPP		tb-DPHP	
Men OPE metabolites		r	p-Value	r	p-Value	r	p-Value	r	p-Value
	BDCIPP	**0.31**	<0.0001	0.03	0.58	0.06	0.41	-0.26	0.11
	DPHP	0.06	0.33	**0.22**	0.0002	**0.14**	0.04	-0.05	0.76
	ip-PPP	0.06	0.42	0.06	0.43	**0.20**	0.01	-0.05	0.81
	tb-DPHP	0.02	0.92	0.09	0.56	0.15	0.44	**0.70**	0.04

Figure IV.1: Adjusted percent change in urinary OPE metabolite concentrations with self-reported PCP use for 230 women from the EARTH cohort.

		OPE Metabolite			
PCP	n	BDCIPP	DPHP	ip-PPP	∑OPE
Deodorant	378	-6	28*	26*	20
Shampoo	367	11	2	2	13
Toothpaste	364	12	48	80	49
Conditioner	323	-11	3	21*	5
Bar Soap	321	-20	-1	1	-16
Hand/Body Lotion	298	-2	6	4	-5
Colored Cosmetics	203	-15	27*	26*	14
Face Moisturizer	146	13	60*	-3	23
Hair Spray/Gel	134	-7	14	21	0
Liquid Soap	128	16	4	2	16
Mouthwash	115	-3	-6	8	9
Cologne/Perfume	114	-8	17	19	13
Other Toiletries	94	-16	2	-13	-21
Other Hair Products	92	13	19	27*	14
Hand Sanitizer	80	-25	5	6	0
Shaving Cream	76	13	6	-2	-21
Suntan /Block Lotion	49	-2	6	4	16
Nail Polish Remover	23	32	77**	21	32
Nail Polish	21	-34	134**	5	52

Heat map for adjusted percent change in PFR metabolite concentrations with self- reported PCP within 24 hr. of urine sample collection (n=638 urine samples). Models are adjusted for Specific Gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; * p<0.05, ** p<0.005

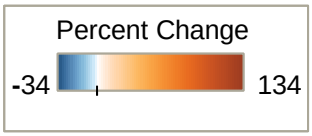


Figure IV.2: Adjusted percent change in urinary OPE metabolite concentrations with self-reported PCP use for 229 men from the EARTH cohort.

		PFR Metabolite			
PCP	n	BDCIPP	DPHP	ip-PPP	∑OPE
Deodorant	211	-10	-19	2	-14
Shampoo	204	-9	-9	23	-6
Toothpaste	190	-10	-16	28	-8
Bar Soap	177	4	-12	12	-4
Shaving Cream	87	-10	-14	1	-10
Mouthwash	79	26	31*	16	31*
Hair Spray/Gel	74	6	16	16	20
Hand/Body Lotion	63	4	-16	-10	-7
Liquid Soap	59	26	-11	-23	-1
Hand Sanitizer	54	-11	-16	-20	-13
Cologne/Perfume	41	34	-4	12	6
Conditioner	40	-4	-8	8	-8
Face Moisturizer	18	-3	-6	1	11
Aftershave	16	7	9	-44	8
Other Hair Products	16	6	14	-11	3
Other Toiletries	14	-6	-7	8	-10
Suntan/Block Lotion	7	30	-32	-35	-1

Heat map for adjusted percent change in OPE metabolite concentrations with self- reported PCP within 24 hr. of urine sample collection (n=335 urine samples). Models are adjusted for Specific Gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; * p<0.05

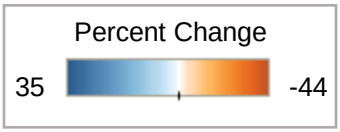


Figure IV.3. Adjusted percent change in urinary OPE metabolite concentrations with self-reported household product use for 230 women from the EARTH cohort.

		PFR Metabolite			
Household Product	n	BDCIPP	DPHP	ip-PPP	∑OPE
Laundry Detergent	147	-4	-10	1	-10
Hand Dishwashing Liquid	123	0.20	-27*	1	-8
Cleaners	99	-3	-6	6	3
Fabric Softener	63	-20	-6	-12	-21
Vinyl Gloves	28	32	13	32	40
Furniture Polish	9	12	-10	-18	-13
Vinyl Boots	8	16	-14	-27	5

Heat map for adjusted percent change in PFR metabolite concentrations with self- reported household product within 24 hr. of urine sample collection (n=638 urine samples). Models are adjusted for Specific Gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; * p<0.05

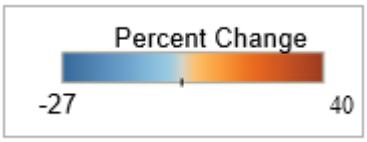
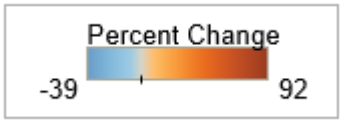


Figure IV.4. Adjusted percent change in urinary OPE metabolite concentrations with self-reported household product use for 229 men from the EARTH cohort.

		PFR Metabolite			
Household Product	n	BDCIPP	DPHP	ip-PPP	∑OPE
Hand Dishwashing Liquid	59	26	0	8	6
Cleaners	38	7	14	12	15
Laundry Detergent	22	31	-24	-2	-2
Vinyl Gloves	9	92	92*	28	68*
Fabric Softener	8	57	63	-14	-34
Paint/Solvent	5	85	-23	-39	-36

Heat map for adjusted percent change in PFR metabolite concentrations with self- reported household product within 24 hr. of urine sample collection (n=335 urine samples). Models are adjusted for Specific Gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; * p<0.05



Chapter V Appendix

Table IV.A.1. Regression coefficients (95% CI) for reported use of personal care products (PCP) and OPE metabolites within 24 hours of urine specimen collection for 230 women from the EARTH cohort.

	OPE Metabolites [a]												
	BDCIPP				DPHP			ip-PPP			∑OPE		
	n [b]	B	95%CI	p	B	95%CI	p	B	95%CI	p	B	95%CI	p
Deodorant	378	-0.06	(-0.33, 0.20)	0.64	**0.25**	(0.05, 0.45)	0.02	**0.23**	(0.01, 0.44)	0.04	0.18	(-0.06, 0.43)	0.14
Shampoo	367	0.10	(-0.15, 0.35)	0.43	0.02	(-0.17, 0.21)	0.85	0.02	(-0.18, 0.23)	0.81	0.12	(-0.11, 0.36)	0.31
Toothpaste	364	0.11	(-1.14, 1.37)	0.86	0.39	(-0.58, 1.36)	0.43	0.59	(-0.43, 1.60)	0.25	0.40	(-1.39, 2.20)	0.66
Conditioner	323	-0.12	(-0.35, 0.12)	0.33	0.03	(-0.15, 0.21)	0.75	**0.19**	(0.01, 0.38)	0.04	0.05	(-0.17, 0.26)	0.67
Bar Soap	321	-0.22	(-0.46, 0.03)	0.08	-0.01	(-0.20, 0.17)	0.89	0.01	(-0.19, 0.20)	0.94	-0.18	(-0.40, 0.04)	0.10
Hand/Body Lotion	298	-0.02	(-0.24, 0.21)	0.88	0.06	(-0.17, 0.23)	0.53	0.04	(-0.14, 0.22)	0.69	-0.05	(-0.25, 0.16)	0.66
Colored Cosmetics	203	-0.16	(-0.38, 0.06)	0.14	**0.24**	(0.07, 0.40)	0.01	**0.23**	(0.05, 0.40)	0.01	0.13	(-0.07, 0.33)	0.21
Face Moisturizer	146	0.12	(-0.37, 0.60)	0.63	**0.47**	(0.14, 0.80)	0.01	-0.03	(-0.37, 0.31)	0.87	0.21	(-0.18, 0.60)	0.29
Hair Spray/Gel	134	-0.07	(-0.31, 0.17)	0.57	0.13	(-0.06, 0.31)	0.18	0.19	(-0.001, 0.39)	0.05	0.004	(-0.21, 0.22)	0.97
Liquid Soap	128	0.15	(-0.14, 0.45)	0.30	0.04	(-0.19, 0.27)	0.72	0.02	(-0.24, 0.27)	0.91	0.15	(-0.13, 0.43)	0.28
Mouthwash	115	-0.03	(-0.31, 0.24)	0.82	-0.06	(-0.27, 0.15)	0.57	0.08	(-0.14, 0.30)	0.46	0.09	(-0.16, 0.35)	0.47
Cologne/ Perfume	114	-0.08	(-0.34, 0.17)	0.53	0.16	(-0.03, 0.36)	0.10	0.17	(-0.04, 0.37)	0.11	0.12	(-0.12, 0.35)	0.33
Other Toiletries	94	-0.18	(-0.62, 0.25)	0.41	0.02	(-0.28, 0.33)	0.88	-0.14	(-0.45, 0.16)	0.35	-0.23	(-0.60, 0.13)	0.21
Other Hair Products	92	0.12	(-0.16, 0.40)	0.39	0.17	(-0.05, 0.38)	0.13	**0.24**	(0.02, 0.46)	0.04	0.13	(-0.11, 0.37)	0.30
Hand Sanitizer	80	-0.29	(-0.59, 0.01)	0.06	0.05	(-0.18, 0.28)	0.67	0.06	(-0.18, 0.30)	0.64	-0.005	(-0.26, 0.25)	0.97

Shaving Cream	76	0.12	(-0.19, 0.44)	0.44	0.06	(-0.18, 0.30)	0.61	-0.02	(-0.27, 0.23)	0.86	-0.23	(-0.50, 0.05)	0.10
Suntan/ Block Lotion	49	0.02	(-0.33, 0.36)	0.92	0.16	(-0.11, 0.42)	0.25	0.13	(-0.15, 0.41)	0.37	0.15	(-0.17, 0.47)	0.35
Nail Polish Remover	23	0.28	(-0.26, 0.81)	0.31	**0.57**	(0.19, 0.95)	0.004	0.05	(-0.33, 0.43)	0.81	0.28	(-0.13, 0.70)	0.17
Nail Polish	21	-0.41	(-0.89, 0.07)	0.10	**0.85**	(0.48,1.21)	<0.001	0.19	(-0.20, 0.59)	0.33	0.42	(-0.06, 0.81)	0.09

[a] Natural log transformation; [b] Reported 'yes' to PCP use within 24 hr. of PFR sample; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of PCP n <5 were not included in analysis

Table IV.A.2. Regression coefficients (95% CI) for reported use of personal care products (PCP) and OPE metabolites within 24 hours of urine specimen collection for 229 men from the EARTH cohort.

	OPE Metabolites [a]												
	BDCIPP				DPHP			ip-PPP			∑OPE		
	n [b]	B	95%CI	p	B	95%CI	p	B	95%CI	p	B	95%CI	p
Deodorant	211	-0.10	(-0.53, -0.33)	0.65	-0.21	(-0.52, 0.09)	0.16	0.02	(-0.30, 0.35)	0.90	-0.15	(-0.42, 0.11)	0.25
Shampoo	204	-0.09	(-0.48, -0.30)	0.64	-0.09	(-0.37, 0.19)	0.53	0.21	(-0.09, 0.50)	0.17	-0.06	(-0.30, 0.19)	0.65
Toothpaste	190	-0.11	(-1.09, 1.31)	0.86	-0.17	(-1.04, 0.70)	0.70	0.25	(-0.70, 1.19)	0.60	-0.08	(-0.85, 0.70)	0.74
Bar Soap	177	0.04	(-0.29, 0.38)	0.79	-0.13	(-0.37, 0.11)	0.28	0.11	(-0.15, 0.36)	0.40	-0.04	(-0.25, 0.17)	0.70
Shaving Cream	87	-0.10	(-0.42, 0.22)	0.53	-0.15	(-0.37, 0.08)	0.21	0.01	(-0.24,0.26)	0.94	-0.10	(-0.30, 0.10)	0.33
Mouthwash	79	0.23	(-0.09, 0.55)	0.16	**0.27**	(0.03, 0.49)	0.03	0.15	(-0.10, 0.39)	0.24	**0.27**	(0.08, 0.48)	0.01
Hair Spray/Gel	74	0.06	(-0.28, 0.39)	0.74	0.15	(-0.09, 0.39)	0.22	0.15	(-0.10, 0.41)	0.24	0.18	(-0.03, 0.38)	0.09
Hand/Body Lotion	63	0.04	(-0.31, 0.39)	0.82	-0.17	(-0.42, 0.08)	0.19	-0.10	(-0.37, 0.17)	0.47	-0.07	(-0.29, 0.15)	0.52
Liquid Soap	59	0.23	(-0.20, 0.66)	0.28	-0.12	(-0.45, 0.22)	0.49	-0.26	(-0.58, 0.07)	0.12	-0.01	(-0.29, 0.28)	0.97
Hand Sanitizer	54	-0.12	(-0.50, 0.26)	0.51	-0.08	(-0.35, 0.20)	0.58	-0.22	(-0.52, 0.07)	0.14	-0.14	(-0.38, 0.11)	0.26
Cologne/ Perfume	41	0.29	(-0.12, 0.70)	0.17	-0.04	(-0.34, 0.25)	0.78	0.11	(-0.21, 0.42)	0.50	0.06	(-0.20, 0.32)	0.63
Conditioner	40	-0.04	(-0.46, 0.38)	0.85	-0.08	(-0.38, 0.22)	0.59	0.08	(-0.24, 0.40)	0.62	-0.08	(-0.34, 0.18)	0.55
Face Moisturizer	18	-0.03	(-0.72, 0.67)	0.94	-0.06	(-0.47, 0.36)	0.78	0.005	(-0.50, 0.51)	0.99	0.10	(-0.30, 0.51)	0.60
Aftershave	16	0.07	(-0.52, 0.66)	0.81	0.09	(-0.34, 0.51)	0.69	-0.42	(-0.87, 0.04)	0.07	0.08	(-0.29, 0.46)	0.66
Other Hair Products	16	0.06	(-0.54, 0.66)	0.85	0.13	(-0.30, 0.57)	0.53	-0.12	(-0.59, 0.34)	0.60	0.03	(-0.35, 0.41)	0.87
Other Toiletries	14	-0.06	(-0.86, 0.75)	0.89	-0.07	(-0.57, 0.43)	0.78	0.08	(-0.53, 0.69)	0.78	-0.11	(-0.58, 0.36)	0.62

Suntan/ Block Lotion	7	0.26	(-0.60, 1.12)	0.56	-0.38	(-1.00, 0.24)	0.22	-0.43	(-1.10, 0.24)	0.20	-0.01	(-0.56, 0.54)	0.98

[a] Natural log transformation; [b] Reported 'yes' to PCP use within 24hrs. of urine sample; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of PCP n <5 were not included in analysis

Table IV.A.3. Regression coefficients (95% CI) for reported use of household products (HP) and OPE metabolites within 24 hours of urine specimen collection for 230 women from the EARTH cohort.

	OPE Metabolites [a]											
	BDCIPP				DPHP			ip-PPP			∑OPE	
	n [b]	B	95%CI	p	B	95%CI	p	B	95%CI	p	B	95%CI	p
Laundry Detergent	147	-0.04	(-0.26, 0.18)	0.74	-0.12	(-0.29, 0.05)	0.17	0.01	(-0.17, 0.19)	0.92	-0.11	(-0.32, 0.10)	0.31
Hand Dishwashing Liq. [c]	123	0.002	(-0.40, 0.40)	0.99	**-0.31**	(-0.59, -0.03)	0.03	0.01	(-0.27, 0.29)	0.96	-0.08	(-0.40, 0.24)	0.63
Cleaners	99	-0.04	(-0.31, 0.23)	0.78	-0.06	(-0.26, 0.15)	0.57	0.06	(-0.15, 0.28)	0.57	0.03	(-0.21, 0.27)	0.79
Fabric Softener	63	-0.22	(-0.54, 0.09)	0.16	-0.04	(-0.28, 0.20)	0.73	-0.13	(-0.38, 0.13)	0.32	-0.23	(-0.52, 0.05)	0.11
Vinyl Gloves	28	0.28	(-0.22, 0.79)	0.27	0.12	(-0.26, 0.50)	0.54	0.28	(-0.12, 0.68)	0.17	0.34	(-0.11, 0.80)	0.14
Furniture Polish	9	0.11	(-0.65, 0.86)	0.78	-0.11	(-0.69, 0.47)	0.72	-0.19	(-0.80, 0.43)	0.55	-0.14	(-0.78, 0.50)	0.66
Vinyl Boots	8	0.15	(-0.64, 0.95)	0.70	-0.15	(-0.77, 0.46)	0.62	-0.31	(-0.95, 0.34)	0.35	0.05	(-0.75, 0.86)	0.90

[a] natural log transformation; [b] Reported 'yes' to HP within 24hrs. of urine sample; [c] n=183; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of HP n <5 were not included in analysis

Table IV.A. 4. Regression coefficients (95% CI) for reported use of household products (HP) and OPE metabolites within 24 hours of urine specimen collection for 229 men from the EARTH cohort.

	OPE Metabolites [a]											
	BDCIPP				DPHP			ip-PPP			∑OPE	
	n [b]	B	95%CI	p	B	95%CI	p	B	95%CI	p	**B**	95%CI	p
Hand Dishwashing Liq. [c]	59	0.23	(-0.32, 0.78)	0.39	0.003	(-0.32, 0.33)	0.98	0.08	(-0.32, 0.48)	0.68	0.06	(-0.26, 0.38)	0.69
Cleaners	38	0.07	(-0.33, 0.48)	0.71	0.13	(-0.16, 0.42)	0.39	0.19	(-0.12, 0.50)	0.23	0.14	(-0.12, 0.39)	0.29
Laundry Detergent [d]	22	0.27	(-0.24, 0.77)	0.30	-0.27	(-0.64, 0.10)	0.15	-0.02	(-0.41, 0.38)	0.94	-0.02	(-0.35, 0.30)	0.90
Vinyl Gloves [d]	9	0.65	(-0.13, 1.42)	0.10	**0.65**	(0.09, 1.20)	0.02	0.25	(-0.35, 0.85)	0.41	**0.52**	(0.03, 1.01)	0.04
Fabric Softener	8	-0.56	(-1.35, 0.22)	0.16	-0.47	(-1.05, 0.11)	0.11	-0.15	(-0.78, 0.47)	0.63	-0.42	(-0.93, 0.09)	0.10
Paint/Solvent [c]	5	-0.16	(-1.31, 0.99)	0.77	-0.27	(-0.99, 0.46)	0.45	-0.49	(-1.34, 0.36)	0.25	-0.44	(-1.08, 0.21)	0.17

[a] natural log transformation; [b] n= reported using household products within 24hrs. of urine sample; [c] n=108; [d] n=247; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of HP n <5 were not included in analysis

Table IV.A. 5. Adjusted percent change (95% CI) for reported use of personal care products (PCP) and PFR metabolites within 24 hours of urine specimen collection for 230 women.

	PFR Metabolites												
	BDCIPP				DPHP			ip-PPP			∑PFR		
	n [a]	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p
Deodorant	378	-6	(-28, 22)	0.64	**28**	(5, 57)	0.02	**26**	(1, 55)	0.04	20	(-6, 54)	0.14
Shampoo	367	11	(-14, 42)	0.43	2	(-16, 23)	0.85	2	(-16, 26)	0.81	13	(-10, 43)	0.31
Toothpaste	364	12	(-68, 294)	0.86	48	(-44, 290)	0.43	80	(-35, 395)	0.25	49	(-75, 803)	0.66
Conditioner	323	-11	(-30, 13)	0.33	3	(-14, 23)	0.75	**21**	(1, 46)	0.04	5	(-16, 30)	0.67
Bar Soap	321	-20	(-37, 3)	0.08	-1	(-18, 19)	0.89	1	(-17, 22)	0.94	-16	(-33, 4)	0.10
Hand/Body Lotion	298	-2	(-21, 23)	0.88	6	(-16, 26)	0.53	4	(-13, 25)	0.69	-5	(-22, 17)	0.66
Colored Cosmetics	203	-15	(-32, 6)	0.14	**27**	(7, 49)	0.01	**26**	(5, 49)	0.01	14	(-7, 39)	0.21
Face Moisturizer	146	13	(-31, 82)	0.63	**60**	(15, 123)	0.01	-3	(-31, 36)	0.87	23	(-16, 82)	0.29
Hair Spray/Gel	134	-7	(-27, 19)	0.57	14	(6, 36)	0.18	21	(-0.01, 48)	0.05	0.01	(-19, 25)	0.97
Liquid Soap	128	16	(-13, 57)	0.30	4	(-17, 31)	0.72	2	(-21, 31)	0.91	16	(-12, 54)	0.28
Mouthwash	115	-3	(-27, 27)	0.82	-6	(-24, 16)	0.57	8	(-13, 35)	0.46	9	(-15, 42)	0.47
Cologne/ Perfume	114	-8	(-29, 19)	0.53	17	(-3, 43)	0.10	19	(-4, 45)	0.11	13	(-11, 42)	0.33
Other Toiletries	94	-16	(-46, 28)	0.41	2	(-24, 39)	0.88	-13	(-36, 17)	0.35	-21	(-45, 14)	0.21
Other Hair Products	92	13	(-15, 49)	0.39	19	(-5, 46)	0.13	**27**	(2, 58)	0.04	14	(-10, 45)	0.30
Hand Sanitizer	80	-25	(-45, 1)	0.06	5	(-16, 32)	0.67	6	(-16, 35)	0.64	-0.1	(-23, 28)	0.97
Shaving Cream	76	13	(-17, 55)	0.44	6	(-16, 35)	0.61	-2	(-24, 26)	0.86	-21	(-39, 5)	0.10

Suntan/ Block Lotion	49	02	(-28, 43)	0.92	17	(-10, 52)	0.25	14	(-14, 51)	0.37	16	(-16, 60)	0.35
Nail Polish Remover	23	32	(-23, 125)	0.31	**77**	(21, 159)	0.004	5	(-28, 54)	0.81	32	(-12, 101)	0.17
Nail Polish	21	-34	(-59, 7)	0.10	**134**	(62, 235)	<0.0001	21	(18, 80)	0.33	52	(-6, 125)	0.09

[a] Reported 'yes' to PCP use within 24 hr. of PFR sample; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of PCP n <5 were not included in analysis

Table IV.A.6. Adjusted percent change (95% CI) for reported use of personal care products (PCP) and PFR metabolites within 24 hours of urine specimen collection for 229 men.

	PFR Metabolites												
	BDCIPP				DPHP			ip-PPP			∑OPE		
	n [a]	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p
Deodorant	211	-10	(-41, 28)	0.65	-19	(-41, 9)	0.16	2	(-26, 42)	0.90	-14	(-34, 12)	0.25
Shampoo	204	-9	(-38, 26)	0.64	-9	(-31, 21)	0.53	23	(-9, 65)	0.17	-6	(-26, 21)	0.65
Toothpaste	190	-10	(-66, 271)	0.86	-16	(-65, 101)	0.70	28	(-50, 229)	0.60	-8	(-57, 101)	0.74
Bar Soap	177	4	(-25, 46)	0.79	-12	(-31, 12)	0.28	12	(-14, 43)	0.40	-4	(-22, 19)	0.70
Shaving Cream	87	-10	(-34, 25)	0.53	-14	(-31, 8)	0.21	1	(-21, 30)	0.94	-10	(-26, 11)	0.33
Mouthwash	79	26	(-9, 73)	0.16	**31**	(3, 63)	0.03	16	(-10, 48)	0.24	**31**	(8, 62)	0.01
Hair Spray/Gel	74	6	(-24, 48)	0.74	16	(-9, 48)	0.22	16	(-10, 51)	0.24	20	(-3, 46)	0.09
Hand/Body Lotion	63	4	(-27, 48)	0.82	-16	(-34, 8)	0.19	-10	(-31, 19)	0.47	-7	(-25, 16)	0.52
Liquid Soap	59	26	(-18, 93)	0.28	-11	(-36, 25)	0.49	-23	(-44, 7)	0.12	-1	(-25, 32)	0.97
Hand Sanitizer	54	-11	(-39, 30)	0.51	-16	(-30, 22)	0.58	-20	(-41, 7)	0.14	-13	(-32, 12)	0.26
Cologne/ Perfume	41	34	(-11, 101)	0.17	-4	(-29, 28)	0.78	12	(-19, 52)	0.50	6	(-18, 38)	0.63
Conditioner	40	-4	(-37, 46)	0.85	-8	(-32, 25)	0.59	8	(-21, 49)	0.62	-8	(-29, 20)	0.55
Face Moisturizer	18	-3	(-51, 95)	0.94	-6	(-37, 43)	0.78	1	(-39, 67)	0.99	11	(-26, 67)	0.60
Aftershave	16	7	(-41, 93)	0.81	9	(-29, 67)	0.69	-44	(-58, 4)	0.07	8	(-25, 58)	0.66
Other Hair Products	16	6	(-42, 93)	0.85	14	(-26, 77)	0.53	-11	(-45, 40)	0.60	3	(-30, 51)	0.87
Other Toiletries	14	-6	(-58, 112)	0.89	-7	(-43, 54)	0.78	8	(-41, 99)	0.78	-10	(-44, 43)	0.62

Suntan/ Block Lotion	7	30	(-45, 206)	0.56	-32	(-63, 27)	0.22	-35	(-67, 27)	0.20	-1	(-43, 72)	0.98

[a] Reported 'yes' to PCP use within 24hrs. of urine sample; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of PCP n <5 were not included in analysis

Table IV.A.7. Adjusted percent change (95% CI) for reported use of household products (HP) and PFR metabolites within 24 hours of urine specimen collection for 230 women (n=479).

	PFR Metabolites [a]											
	BDCIPP				DPHP			ip-PPP			∑OPE	
	n [b]	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p
Laundry Detergent	147	-4	(-23, 20)	0.74	-10	(-25, 5)	0.17	1	(-16, 21)	0.92	-10	(-27, 11)	0.31
Hand Dishwashing Liq. [c]	123	0.20	(-33, 49)	0.99	**-27**	(-45, -3)	0.03	1	(-24, 34)	0.96	-8	(-33, 27)	0.63
Cleaners	99	-3	(-27, 26)	0.78	-6	(-23, 16)	0.57	6	(-14, 32)	0.57	3	(-19, 31)	0.79
Fabric Softener	63	-20	(-42, 9)	0.16	-6	(-24, 22)	0.73	-12	(-32, 14)	0.32	-21	(-41, 5)	0.11
Vinyl Gloves	28	32	(-20, 120)	0.27	13	(-23, 65)	0.54	32	(-11, 97)	0.17	40	(-10, 123)	0.14
Furniture Polish	9	12	(-48, 136)	0.78	-10	(-50, 60)	0.72	-18	(-55, 54)	0.55	-13	(-54, 65)	0.66
Vinyl Boots	8	16	(-47, 159)	0.70	-14	(-54, 58)	0.62	-27	(-61, 40)	0.35	5	(-53, 136)	0.90

[a] Reported 'yes' to HP within 24hrs. of urine sample; [b] n=183; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of HP n <5 were not included in analysis

Table IV.A.8. Adjusted percent change (95% CI) for reported use of household products (HP) and PFR metabolites within 24 hours of urine specimen collection for 229 men (n=248).

	PFR Metabolites [a]											
	BDCIPP				DPHP			ip-PPP			∑PFR	
	n [a]	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p	% change	95%CI	p
Hand Dishwashing Liq. [b]	59	26	(-27, 118)	0.39	0	(-27, 39)	0.98	8	(-27, 62)	0.68	6	(-23, 46)	0.69
Cleaners	38	7	(-28, 62)	0.71	14	(-15, 52)	0.39	12	(-11, 65)	0.23	15	(-11, 48)	0.29
Laundry Detergent [c]	22	31	(-21, 116)	0.30	-24	(-47, 11)	0.15	-2	(-34, 46)	0.94	-2	(-30, 35)	0.90
Vinyl Gloves [c]	9	92	(-12, 314)	0.10	**92**	(9, 232)	0.02	28	(-30, 134)	0.41	**68**	(3, 175)	0.04
Fabric Softener	8	57	(-74, 25)	0.16	63	(-65, 12)	0.11	-14	(-54, 60)	0.63	-34	(-61, 9)	0.10
Paint/Solvent [b]	5	85	(-73, 169)	0.77	-23	(-63, 58)	0.45	-39	(-74, 43)	0.25	-36	(-66, 23)	0.17

[a] n= reported using household products within 24hrs. of urine sample; [b] n=108; [c] n=247; Models adjusted for Specific gravity (SG), age, BMI, race (other/white), smoking status (never/ever), education & season; Reported use of HP n <5 were not included in analysis

CHAPTER V

AIM 3 Part B: The Association Between Urinary Concentrations of Organophosphate Ester Metabolites and Semen Parameters Among Men from a Fertility Clinic

Abstract

Background: The use of organophosphate esters as flame retardants has steadily increased as brominated compounds have been or are being phased out. OPEs have half-lives from hours to days, yet human exposure is widespread. Animal studies have shown adverse impacts on male reproduction, but human data are lacking. The objective of this study was to investigate the associations between urinary concentrations of OPE metabolites and semen parameters.

Methods: A subset of 220 men from an existing longitudinal cohort of couples was recruited from Massachusetts General Hospital fertility clinic between 2005 and 2015. Men having a female partner currently in the study and providing at least one urine sample for OPE measurement during an *in vitro* fertilization (IVF) cycle were eligible. Semen parameters included sperm count, concentration, motility, and morphology; some men had samples measured from multiple clinic visits (up to five visits; n=269 semen samples). Metabolites [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) and bis(1-chloro-2-propyl) phosphate (BCIPP)] were measured in

urine samples (between one and five urine samples per participant; n=355 urine samples; n=83 participants >1 urine sample). Semen parameters were evaluated continuously and dichotomized for models. Metabolites were assessed for associations with semen parameters as continuous and categorized into quartiles using multivariable generalized mixed models, adjusted for specific gravity, age, BMI, smoking, and abstinence period.

Results: Metabolites BDCIPP, DPHP, and ip-PPP were detected in a high proportion of urine samples (85%, 86%, and 65% respectively). Concentrations varied by season of collection, particularly for BDCIPP where samples collected in the summer were approximately 2-fold higher than concentrations of other seasons (p-value<0.0001). The odds of having a sperm count less than 39 mil/ejaculate decreased by 20% for increasing BDCIPP concentrations (p-value=0.04). When regressing semen parameters on OPE metabolite quartiles, some negative associations were observed for individual quartiles among sample volume and morphology, but overall associations were weak and inconsistent.

Conclusion: Detection rates were high for BDCIPP, DPHP, and ip-PPP. We did not observe consistent associations between OPE metabolites and semen parameters. Due to the high prevalence of exposure, further investigation of other potential health effects should be conducted.

Introduction

Infertility, the inability to conceive after one year of unprotected intercourse, affects approximately one out of every six couples [1]. In 2002, a national survey estimated two million couples in the US suffer from infertility [2]. An increase in infertility is partially related to the postponement of first birth [3,4]. However, aside from advanced age, genetic risk factors, psychosocial factors, and environmental agents can also impair fertility [5,6].

The underlying cause of infertility may be related to female or male factors or a combination of both. In 2002, approximately 20% of male participants in the National Survey of Family Growth (NSFG) reported fertility problems in the US [7]. However, this estimate is likely modest as only men from couples struggling to conceive will seek fertility evaluation, thus male factor infertility is thought to be underdiagnosed [7,8]. In 2002, the cost of male factor infertility alone was $17 million US dollars, which does not include the additional $18 billion for assisted reproductive technology treatment [1].

To date, a semen analysis measuring sperm count, concentration, motility, morphology, and volume remains the primary evaluation for male factor infertility [8,9]. A recent global meta-analysis found an approximate 50% reduction in total sperm count and sperm concentration among men from Western countries over the last several decades, regardless of fertility diagnosis [10]. Semen quality is also associated with other various health outcomes. A study of Finnish men found an increase risk in testicular cancer among those with poor semen quality [11], while a Danish study found subpar semen associated with a shorter life span [12]. Many environmental agents such as glycol ethers, pesticides, and phthalates are also known to impact semen quality [6].

Among possible environmental chemicals of concern for reproductive health are organophosphate esters (OPEs), which are increasingly being used as flame retardants (FRs). The use of OPEs has grown due to their use as replacement chemicals for the phased-out of polybrominated diphenyl ethers (PBDEs). As their prevalence rose, OPEs became and remain a high production volume chemical. Today they are commonly applied to materials for use as either a flame retardant, or as a plasticizer, therefore are common in polyvinyl chloride (PVC), hydraulic fluids, and polyurethane foam (PUF) in cars and furniture [13–15]. OPE s include both chlorinated alkyl esters such as tris(2-chloroisopropyl) phosphate (TCIPP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP), and non-halogenated aryl phosphates such as triphenyl phosphate (TPHP) [13,16]. Often considered 'additive' compounds, the weak bonds allow volatilization into air and settlement in dust. OPE s have been detected in the dust of homes, cars, and offices [16,17]. Unlike brominated flame retardants, OPEs are considered non-persistent, with a short half-life in humans ranging from several hours to days, yet they are detected in nearly 100% of urine samples from men [18], pregnant women [19] , and children [20].

To date, studies assessing the health effects of OPEs are limited, yet animal and *in vitro* studies suggest these compounds act as endocrine disrupting chemicals. A study of TPHP and tris(2-chloroethyl) phosphate (TCEP) in mice found a disruption of gene expression for testosterone synthesis and oxidative stress [21], while an *in vitro* study of mouse Leydig cells found a disruption in steroid production [22]. A small study of US men detected inverse relationships of bis(1,3-dichloropropyl) phosphate (BDCPP)

and diphenyl phosphate (DPHP) concentrations in urine with sperm concentration and motility [23]. To the best of our knowledge, this prior analysis is the only human study to date to assess the relationship of OPE s with semen parameters. In our present work, we expand upon this preliminary evidence with a larger cohort to characterize the relationship between five OPE metabolites: bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) with semen parameters in men attending a fertility center.

Methods

*Participant Recruitmen*t

Participants from this analysis are a subset of men from the Environment and Reproductive Health (EARTH) study, a larger cohort assessing the impact of environmental agents on reproductive health. Participation and recruitment have been described elsewhere [24]. Briefly, men (18-54 years of age) attending the Massachusetts General Hospital fertility clinic between 2005 and 2015 were eligible. Participants originated from couples whose infertility diagnosis was either male factor, female factor, or a combination of both. Along with having a female partner enrolled in the EARTH study, men must also have provided at least one urine sample for OPE measurement during an *in vitro* fertilization (IVF) cycle. Thus, men who provided more than one sample were part of a couple who may have had more difficulty achieving pregnancy. Prior vasectomy or hormone supplementation were the only exclusion criteria. Informed

consent was signed by each participant and Institutional Review Board approval was received by all institutions.

Semen Collection and Analysis

Semen collection and analysis have been previously described [24,25]. Briefly, men abstained from ejaculation for 48 hours prior to sample collection into plastic specimen cup. Men provided up to five samples depending on the number of fertility treatments, additional fertility evaluation, or a combination of both. An andrologist quantified sample volume (mL) with a graduated pipet. Sperm concentration (mil/mL) and motility (% motile) was determined using a computer-aided semen analyzer (CASA, version 10 HTM-IVOS; Hamilton Thorne Research, Beverly, MA). Samples (5 µL) were collected on a disposable Leja Slide (Spectrum Technologies, CA, USA) and placed into a pre-warmed (37°C) counting chamber (Sefi-Medical Instruments, Haifa, Israel) before assessing concentration and motility. Among each sample, at least 200 sperm cells were analyzed from four different fields. Progressive motility was graded in accordance to the WHO's assessment criteria of active movement (linearly or in a large circle), regardless of velocity (WHO, 2010). The product of sperm concentration and sample volume determined sperm count (mil/ejaculate) while progressive motility count (mil/ejaculate) was calculated by multiplying progressive motility and total sperm count. Fresh semen samples were allowed to dry on two prepared slides and prepared for morphology (% normal) assessment with a microscope using an oil-immersion 100x objective (Nikon, Tokyo, Japan). A minimum of 200 cells per slide were analyzed for each specimen. Classification of normal or subnormal morphology was determined using strict Kruger scoring criteria [26]. Quality assurance and control procedures in the

laboratory were conducted for sperm morphology smears weekly, as well as quarterly and biannual evaluations for technicians.

Urine Collection and Analysis

Urine samples (from up to five cycles) were collected in sterile polypropylene cups on the day of semen sample collection. Most men provided one urine sample (n=220), while 83 provided two samples and 26 provided three (n≤4 provided four or more samples). Prior to being frozen (-80°) and stored, specific gravity (SG) was measured using a handheld refractometer (National Instrument Company, Inc., Austin, TX). For metabolite analysis, samples were shipped overnight on dry ice to Dr. Stapleton's lab at Duke University (Durham, NC).

Analytic methods for metabolites: BCIPP, BDCIPP, DPHP, ip-PPP, and tb-PPP have been previously described [27]. Briefly, 5 ml aliquots were thawed and transferred to test tubes and spiked with internal standards (d_{10}-BDCIPP = 80 ng, d_{10}-DPHP = 60 ng) before being acidified (pH <6.5) with formic acid and diluted with 1:1 with water. Solid phase extraction (SPE) was used to concentrate and clean samples before drying via nitrogen stream and spiked with the recovery standard ($^{13}C_2$-DPHP = 81.5 ng). Extracts were analyzed using negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS) detailed previously [27]. Optimal parameters under multiple reaction conditions were used to acquire data. The internal standard used for BCIPP and BDCIPP was d_{10}-BDCIPP, while quantification of DPHP, ip-DPHP, and tb-DPHP was performed using d_{10}-DPHP.

Quality assurance and control procedures for LC-MS/MS have been described previously [28]. Briefly, samples were processed in multiple batches including five blanks per batch (5 ml Milli-Q water); each batch providing a distinct method detection limit (MDL). MDLs were designated as three times the standard deviation of laboratory blanks and ranged from: 0.07-0.17 pg/ml for BCIPP, 0.02-0.11 pg/ml for BDCIPP, 0.09-0.18 pg/ml for DPHP, 0.06-0.12 pg/ml for ip-PPP, and 0.04-0.15 pg/ml for tb-PPP. Urine samples from previous studies were pooled to establish a standard reference material (SRM) and routinely analyzed. Duplicates of two-subsamples were analyzed to evaluate precision.

Statistical Analysis

Descriptive statistics for OPE metabolites, semen parameters, and demographic factors were calculated. Values below MDL for metabolites were imputed as MDL/$\sqrt{2}$. Metabolites were presented as uncorrected and adjusted for SG as: $C_{SG} = C*[(SG_M-1)/(SG_i -1)]$, where C_{SG} = SG-adjusted urinary metabolite concentration, C = urinary metabolite concentration, SG_M = mean SG for the population, and SG_i = SG for an individual sample [29]. We evaluated bivariate associations among OPE metabolites, semen parameters, and demographic factors using Spearman correlation coefficients, Wilcoxon rank-sum tests, and Kruskal-Wallis tests as appropriate. An aggregate variable (∑OPE) was imputed by summing the urinary molar concentrations for metabolites BDCIPP, DPHP, and ip-PPP. Intraclass correlation coefficients (ICC) and 95% confidence intervals for metabolites (uncorrected and SG corrected) and semen parameters were calculated to assess variability between samples of each participant. All metabolites and semen parameters presenting as right-skewed were transformed by

natural logarithm for further statistical modeling. OPE metabolites were evaluated as continuous variables and quartiles except for tb-PPP with low detection rate (11.34 %) was modeled as detect/non-detect. Sperm parameters were evaluated both continuously and dichotomized using WHO reference level for sperm: count (< 39mil/ejaculate), concentration (< 15 mil/mL), motility (< 40%), progressive motility (<32%), and morphology (< 4% normal) (WHO, 2010). Initially, crude associations were calculated among OPE metabolites and semen parameters (Table V.A.1). Bivariate tests for possible covariates: age (n=2 missing), BMI (n=3 missing), abstinence period (n=71 missing), race (n=2 missing), smoking status (n=2 missing), education (n=53 missing), and season of collection (n=0 missing) along with biological plausibility and a priori knowledge were used to select covariates for modeling (Table V.A 2) [24,30,31]. Potential confounding was defined as a 10% change in the effect estimate of OPE metabolites on semen parameters and investigated through covariates in Table V.A.2. Although season of sample collection was associated with OPE metabolites, it was not associated with semen parameters, fit criteria for confounding, or found to be an effect modifier, thus not included in final models. Multivariable regression models, adjusted for SG, age, BMI, and abstinence period, were used to test associations using only the first urine and semen sample for all participants (data not shown). Multivariable generalized mixed models with random intercepts using continuous, dichotomous, and quartiles for OPEs were used to assess associations with repeated exposures and/or semen parameters. These models are optimal for IVF cohorts with multiple outcomes per person by accounting for within person correlations as well as account for an unbalanced study design (i.e. different number of cycles per person) when the

imbalance is not random (i.e. more cycles indicate increased difficulty achieving pregnancy). Models included a fixed compound symmetry covariance structure. Continuous outcomes were analyzed using a normal distribution and identity link function while a binary distribution and logit link function was used for dichotomous outcomes. To test for trends, the natural log transformed median urinary concentrations for each quartile was treated as a continuous variable in regression models. We conducted a sensitivity analysis excluding SG measurements below 1.01 and above 1.03 for multivariable models (Table V.A.3) to examine any effect of extreme urine concentrations. Missing data were excluded from models. All statistical analyses were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Study Population

Demographic characteristics of our subsample from the EARTH study are displayed in Table V.1. Demographics from this sample are similar to previous studies in similar cohorts [24] as well as national trends of men undergoing IVF [7] regarding age (mean=36.7 ± 5.1), BMI (27.2 ± 4.0), race or ethnicity (89% white), and education (80% college graduates).

The distribution of semen parameters among our sample is presented in Table 2. All parameters met WHO guidelines for normal sperm among more than half of participants (WHO, 2010). Total sperm count and concentration exceeded the lower limit for normal sperm (43.4 and 15.9, respectively) in 90% of samples. Conversely, less than half of participants had above average motility (45%) while 75% exceeded the

guideline for morphology (4%). Participants provided semen (n=269) and urine (n=355) samples from up to five clinic visits, with the majority of men providing one to three. Repeated measures of semen parameters had moderately strong intraclass correlations (0.51-0.58), except motility (ICC=0.79) and progressive motility (ICC=0.71) which had stronger ICCs (Table V.A.4).

Distributions of OPE metabolites, as both uncorrected and SG-corrected are displayed in Table IV 3. Metabolites BDCIPP, DPHP, and ip-PPP were detected in a high proportion of urine samples (85%, 86%, and 66% respectively). We identified weak ($r < 0.30$) yet significant ($p < 0.01$) correlations among BDCIPP, DPHP, and ip-PPP, and moderate ($r=0.43$) correlations between DPHP and tb-DPHP ($p=0.01$) (Data not shown). Similarly, temporal stability between metabolite measurements (Table V.4) were weak-to-moderate (ICC <0.35) and decreased further when excluding non-detects and adjusting for SG, except for ∑OPE ($r=0.67$). Concentrations varied by season of collection, particularly for BDCIPP where samples collected in the summer had the highest concentrations ($p<0.0001$) (Table IV.A.2).

When modeling OPE metabolites and semen parameters as continuous variables, there were no significant effect estimates from repeated measure models (Table V.5). Whereas when semen parameters were dichotomized, elevated BDCIPP was associated with a decreased odds of low sperm count (OR=0.79, 95% CI=0.64, 0.99; $p=0.04$). Results were similar in a sensitivity analysis excluding extreme urine dilution concentrations ($0.01 \leq SG \leq 1.03$) (Table V.A.3). When modeling OPE metabolites as quartiles, we identified a modest nonlinear trend ($p=0.05$) in progressive motility and ∑OPE (Table V.6). The second quartile for DPHP ($p=0.04$) was positively

associated with sample volume, whereas the third quartile of ∑OPE (p=0.03) concentrations was inversely associated (Table V.6). Semen concentration was positively associated with ∑OPE concentrations (Q3; p=0.04) (Table V.6). DPHP concentrations between 0.66-1.21 µg/L (Quartile 3, p= 0.02) increased the odds of abnormal semen morphology (Table V.A.5). However, overall p-trends were not statistically significant.

Discussion

Although exposure was prevalent, overall, we did not observe consistent associations between OPE metabolites and semen parameters. To our knowledge, this is the largest study to evaluate the relationship between OPE metabolites and semen quality. Most semen parameters in our sample were above established reference levels (WHO, 2010) and within-participant reliability was moderate-to-strong for repeated samples. Metabolites BDCIPP, DPHP, and ip-PPP were detected at high rates in urine and temporal reliability of repeated samples within participant was weak-to-moderate. While we found a decreased odds of a low sperm count (<39 mil/ejaculate) with increasing BDCIPP concentrations, overall associations were weak and inconsistent.

Comparisons with other studies

To date, there are limited studies examining the potential for adverse health effects related to OPE exposure despite their high detection in various environmental media and respective metabolites in urine. Parent compounds TDCPP and TPHP were detected in nearly all samples of house dust from a previous sample of 50 men from the EARTH cohort [32]. Similarly, a study in Durham, North Carolina (n=40 adults)

detected parent compounds to BDCIPP, BCIPP, and DPHP (TDCIPP, TCIPP, and TPHP, respectively) in 100% of samples using silicone wrist bands and >95% of hand wipes [33].

Studies characterizing OPEs in male populations are insufficient compared to those among women and children. Yet, analogous to high detection rate in environmental media, metabolites BDICPP and DPHP were detected in >90% of individuals [18] and >95% of pooled samples [31]. Concentrations of BDCIPP in our samples were six-fold higher compared to a prior study (n=16) of adults in California (Median=0.09 ng/mL) [34]. Our samples of DPHP were also twice as high (Median= 0.44 ng/mL), yet both were similar in having low detection of BCIPP. A small sample (n=29) of office workers in Boston, MA also had slightly lower concentration of BDCIPP (SG-adjusted Mean=408 pg/mil) [35]. However, distributions of BDCIPP, DPHP, and ip-PPP (GM= 0.64, 0.77, 0.32 µg/L respectively) were similar to a recent study of 211 females (n=563 samples) from the EARTH cohort (SG-adjusted GM= 0.66, 0.78, 0.22 µg/L respectively) [28] , yet considerably lower compared to more recent samples of pregnant women in Durham, North Carolina (uncorrected GM 1.8, 1.4, 6.8 µg/L respectively) [30] and Shanghai, China (BDCIPP GM=1.2, DPHP GM=1.1 ng/mL) [36]. Exposure biomarker concentrations of these chemicals may follow a temporal trend, as our OPE concentrations are higher than prior studies, yet similar to samples collected during similar time periods; a recent study combining several cohorts from various parts of the US found a 15-fold increase in BDCIPP samples collected in 2015 compared to those collected in 2002 [37].

The weak to moderate stability in repeated measurements in our sample was somewhat lower than reported from a previous study for BDICPP (ICC=0.55-0.72) and DPHP (ICC=0.35-0.51), although the sample period was considerably shorter (3 months) compared to the present analysis (3 months-5 years) [18]. However, another study with a longer time period (12 months) found ICCs of DPHP to be somewhat lower and similar to our findings (ICC range 0.13-0.39) [38]. We identified a relationship with OPE concentrations and season of sample collection, where concentrations of BDCIPP ($p<0.0001$) were highest in summer (June-August), while DPHP ($p=0.05$) concentrations were highest in the winter (December-February). A sample of adults spanning the US observed a similar seasonal relationship as BDCIPP concentrations in summer were 4.13 times higher than winter samples and contrary to our observations, DPHP concentrations were also highest in summer [37]. Similar results were found among a sample of pregnant women, where summer (June-August) concentrations of BDCIPP and DPHP were almost 4-fold and 60% higher, respectively compared to winter samples [30]. Thus, warmer climates may potentially impact OPE exposure and explain the difference in biomarker levels in our study (Boston, MA) compared to the warmer climates of studies in North Carolina and Shanghai, China [30,36].

Limited research has been conducted on OPE metabolites and male reproductive health. However, we previously reported a decrease in sperm morphology (36%), straight-line velocity (18%), and curvilinear velocity (14%) in association with BDCIPP in a previous study of men (n=33) from the EARTH cohort. The same study also reported decreased sperm concentration (57%) and straight-line velocity (19%) in association with urinary DPHP [23]. Similar relationships were detected in a study (n=50)

of their parent compounds in house dust where concentrations of TDCPP and TPHP were inversely associated with sperm concentration, motility, and morphology, although only the relationship between TPHP and sperm concentration was statistically significant (p=0.01) [32]. In this more robust analysis, we observed suggestive declining trends in our adjusted models among BDCIPP and DPHP with total sperm count and sample volume when modeled as continuous variables. Our observations are inconsistent with previous work, possibly as a result of substantial sample size differences.

Animal and in-vitro studies

Laboratory studies assessing the reproductive impacts of OPEs are also limited, yet suggest OPEs act as endocrine disruptors and induce oxidative stress, both which have been associated with decreased sperm parameters [24,39]. Several *in vitro* models found TDCPP to be an estrogen agonist [40,41] while another found DPHP to have stronger estrogenic activity compared its parent compound (TPHP)[42]. A study of mouse Leydig cells concluded TPHP failed to disrupt steroidogenesis, although increased TPHP concentrations resulted in a 1.7 fold increase in superoxide production [22]. However, another study of mice found TCPP and TECP to alter antioxidant enzymes and testosterone levels [21]. Thus, although animal studies are limited, they suggest OPEs may potentially impact male fertility by altering reproductive hormones and impairing spermatogenesis via the hypothalamic-pituitary-gonadal (HPG) axis, or by disrupting the reactive oxygen species-antioxidant balance necessary for spermatogenesis.

Limitations

Although novel, our study is not without limitations. While the largest study to date, our sample size is somewhat modest. Few prior studies have assessed the predictors of OPE exposure in male populations, and our limited findings are potentially a result of parsimonious modeling combined with residual confounding. Also, isolated exposure to OPEs among our sample is unlikely and simultaneous environmental exposures found to impact semen quality should be considered in future studies [43,44]. Contrary to PBDEs which have a long half-life, OPEs are less-persistent and samples may be subject to more exposure misclassification since urinary metabolite levels may reflect exposure only hours or days prior to sample collection and we were unable to capture the time of urine sample collection. However, we attempted to reduce this source of error by collecting up to five urine samples per participant and previously reported levels remain moderately stable over a three month period [18].

Men from a fertility clinic are a selective population that potentially limit their generalizability to the men from general population [7]. However, the semen quality of these men is comparable with the semen quality of men from the general population. Due to limited studies characterizing OPEs in male cohorts, we are unable to conclude the OPE concentrations found in our study do not reflect levels in the general population or that men from a fertility clinic would respond differently to OPE exposure. Finally, while we analyzed five commonly used OPE metabolites, there are other metabolites such as bis-2chloroethyl phosphate (BCEtP), di-n-butyl phosphate (DBNP), di-benzyl phosphate (DBzP), and di-cresyl phosphate (DCP) that should be the focus of future investigations. Thus, our results are not applicable to all OPE metabolites.

Conclusion

The results from our study characterizing the relationship between OPEs and male reproductive health are inconclusive. Although our findings were inconsistent, we observed high detection of metabolites which coincides with previous and concurrent studies. In comparison to our results and other studies, concentrations of OPE metabolites appear to be increasing over time. Widespread detection rates, temporal increases in concentrations, along with evidence from animal research establish the necessity for additional investigation of OPEs on male reproductive health.

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Table V.1. Demographic characteristics among 220 men from the Environment and Reproductive Health (EARTH) cohort.

Characteristic	N	Mean or %	SD	Median	25^{th}, 75^{th} quartiles
Age	218	36.7	5.1	36.0	32.9, 39.9
BMI	217	27.2	4.0	26.9	24.3, 29.1
Abstinence period	186	3.9	13.9	2.4	1.8, 3.0
Race					
White	194	89.0			
Black	4	1.8			
Asian	13	6.0			
Other	7	3.2			
Smoking Status					
Never smoke	153	70.2			
Past smoker	52	23.9			
Current smoker	13	6.0			
Education					
<High school	3	1.7			
HS grad	6	3.3			
1 or 2 yr. college	12	6.6			
3 or 4 yr. college	11	6.0			
College grad.	61	33.5			
Graduate degree	89	48.9			
Season of sample					
Winter	79	23.5			
Spring	79	23.6			
Summer	90	26.9			
Fall	87	26.0			

SD: standard deviation; Missing: age, race, smoking n=2; BMI n=3; Abstinence period n=34; Education n=38; Season: n=335 using all observations; Winter: December-February, Spring: March-May; Summer: June-August; Fall: September-November

Table V.2. Distribution of semen parameters among 220 men from the EARTH cohort contributing 1-5 samples.

			Percentiles						
Semen Parameter	n	Mean	10th	25th	50th	75th	90th	95th	Max
Total sperm count(mil/ejaculate)	235	172.5	43.4	68.0	133.3	224.8	364.5	511.3	679.1
Concentration (mil/mL)	237	77.02	15.9	30.2	58.2	110.1	140.5	156.5	617.4
Motility (P+NP) (%)	237	44.30	10	25	45	63	75	80	93
Progressive motility (%)	234	24.89	5	12	24	36	45	49	69
Morphology (% normal sperm)	255	6.18	2	4	6	8	10	12	18
Sample volume (mL)	267	2.68	1.0	1.7	2.5	3.5	4.5	5.1	8.7

P+NP: Progressive + Non progressive semen motility

Table V.3. Distribution of uncorrected and Specific gravity corrected OPE metabolites (µg/L) of 220 men from the EARTH cohort (n=355 urine measurements).

				Percentiles					
Uncorrected	N >MDL, (%)	GM	(95% CI)	25th	50th	75th	90th	95th	Max
BDCIPP	285 (85.1)	0.62	(0.55, 0.71)	0.33	0.60	1.38	2.72	3.57	10.30
DPHP	289 (86.3)	0.78	(0.71, 0.87)	0.42	0.74	1.31	2.47	4.13	10.57
ip-PPP	223 (66.6)	0.35	(0.32, 0.39)	<MDL	<MDL	0.62	0.93	1.42	4.56
tb-DPHP	38 (11.3)	0.16	(0.12, 0.20)	<MDL	<MDL	<MDL	0.38	0.73	2.24
SG corrected									
BDCIPP	285 (85.1)	0.64	(0.57, 0.73)	0.35	0.61	1.14	2.37	4.26	20.24
DPHP	289 (86.3)	0.77	(0.70, 0.84)	0.46	0.70	1.15	2.38	3.59	15.55
ip-PPP	223 (66.6)	0.32	(0.29, 0.35)	<MDL	<MDL	0.51	0.84	1.14	4.08
tb-DPHP	38 (11.3)	0.17	(0.12, 0.23)	<MDL	<MDL	<MDL	0.50	1.58	1.87
Specific gravity	-	0.017	(0.016, 0.018)	1.011	1.018	1.024	1.027	1.028	1.038

MDL: Method detection limit; GM: Geometric mean; BCIPP data not shown (n=3 measurements)

Table V.4. Intraclass correlation coefficients (95% CI) for uncorrected and SG corrected repeated urinary OPE metabolites (μg/L) among men from the EARTH cohort.

	All samples [a]				Excluding non-detects			
OPE Metabolite	Uncorrected		SG corrected		Uncorrected		SG corrected	
BDCIPP	0.34	(0.20, 0.51)	0.21	(0.08, 0.45)	0.30 [b]	(0.16, 0.50)	0.18 [b]	(0.05, 0.47)
DPHP	0.07	(0.00, 0.68)	0.09	(0.01, 0.62)	0.07 [a]	(0.00, 0.68)	0.06 [c]	(0.00, 0.81)
ip-PPP	0.37	(0.24, 0.52)	0.25	(0.11, 0.49)	0.28 [d]	(0.13, 0.48)	0.13 [d]	(0.02, 0.55)
∑OPE	0.17	(0.06, 0.42)	0.16	(0.03, 0.50)	0.13 [e]	(0.00, 0.87)	0.67 [e]	(0.45, 0.82)

[a] n=335 samples from 220 men; [b] n=285 samples from 187 men; [c] n=289 samples from 200 men; [d] n=233 samples from 167 men; [e] n=192 samples from 145 men; Men contributing: 1 urine sample n=220, 2 urine samples n=83, 3 urine samples n=26, ≥4 urine samples n=4

Table V.5. Regression coefficients and odds ratios (95% CI) for semen parameters of men from the EARTH cohort contributing 1-5 urine samples.

	OPE Metabolites [a]											
	BDCIPP			DPHP			ip-PPP			∑OPE		
Sperm Parameter	B	95%CI	p-Value	B	95%CI	p-Value	B	95%CI	p-Value	B	95%CI	p-Value
Total sperm count (mill) [a, b]	-0.02	(-0.10, 0.07)	0.70	-0.01	(-0.11, 0.09)	0.82	-0.04	(-0.14, 0.07)	0.52	0.001	(-0.12, 0.12)	0.99
Concentration (mil/mL) [a, c]	0.002	(-0.08, 0.08)	0.96	0.005	(-0.09, 0.10)	0.92	-0.004	(-0.10, 0.09)	0.94	0.02	(-0.09, 0.14)	0.67
Motility (P+NP) (%) [a, c]	0.005	(-0.07, 0.08)	0.90	0.04	(0.05, 0.13)	0.40	0.12	(-0.08, 0.11)	0.72	0.06	(-0.08, 0.20)	0.38
Progressive motility [a, d]	0.04	(-0.04, 0.12)	0.30	0.03	(-0.06, 0.12)	0.53	0.05	(-0.04, 0.14)	0.28	0.06	(-0.05, 0.17)	0.28
Morphology (%norm) [e]	0.17	(-0.16, 0.50)	0.30	0.12	(-0.31,0.55)	0.58	0.21	(-0.21, 0.64)	0.32	0.18	(-0.30, 0.67)	0.45
Sample volume (mL) [f]	-0.05	(-0.18, 0.08)	0.45	-0.08	(-0.24, 0.08)	0.31	-0.04	(-0.20, 0.12)	0.61	-0.12	(-0.31, 0.06)	0.19
Odds Ratio	OR			OR			OR			OR		
Total sperm count <39 mil/ejaculate [b]	**0.79**	(0.64, 0.99)	0.04	0.93	(0.55, 1.55)	0.78	1.04	(0.63, 1.72)	0.88	0.76	(0.48, 1.20)	0.39
Sperm concentration <15 mil/mL [c]	0.90	(0.72, 1.13)	0.37	0.97	(0.60, 1.57)	0.90	0.92	(0.54, 1.57)	0.76	0.83	(0.51, 1.35)	0.52
Percent motile sperm (P+NP) <32 [g]	1.07	(0.86, 1.35)	0.53	1.13	(0.84, 1.51)	0.42	1.04	(0.79, 1.38)	0.78	1.09	(0.78, 1.52)	0.60

Percent motile sperm (P+NP) <40 [c]	1.05	(0.84, 1.31)	0.63	1.01	(0.76, 1.35)	0.92	0.90	(0.66, 1.21)	0.48	0.93	(0.67, 1.29)	0.66
Percent morph. Sperm <4 [e]	0.92	(0.70, 1.20)	0.53	1.14	(0.83, 1.57)	0.44	0.94	(0.68, 1.30)	0.71	0.97	(0.66, 1.42)	0.86

[a] natural log transformation; [b] n=230; [c] n=232; [d] n=229; [e] n=249; [f] n=261; [g] n=263; Models adjusted for specific gravity, age, BMI, smoking status & abstinence period

Table V.6. Regression coefficients (95% CIs) by quartile of OPE metabolite for men from the EARTH cohort contributing 1-5 urine samples.

OPE [a] (quartile range)	Semen Parameters											
	Total sperm count [a]		Concentration (mil/mL) [a]		Motility (P+NP) (%) [a]		Progressive motility [a]		Morphology (% norm)		Sample volume (mL)	
BDCIPP												
Q1 (0.2-0.17)	-		-		-		-		-		-	
Q2 (0.18-0.51)	-0.11	(-0.38, 0.16)	-0.08	(-0.32, 0.17)	0.01	(-0.23, 0.24)	0.02	(-0.22, 0.25)	-0.90	(-1.94, 0.14)	-0.21	(-0.60, 0.18)
Q3 (0.52-1.11)	0.12	(-0.91, 1.14)	-0.31	(-1.23, 0.62)	0.42	(-0.48, 1.31)	-0.04	(-0.94, 0.86)	-1.29	(-5.27, 2.69)	-0.83	(-0.68, 2.34)
Q4 (1.12-10.30)	0.06	(-0.44, 0.56)	0.05	(-0.41, 0.51)	0.15	(-0.29, 0.60)	0.29	(-0.16, 0.75)	1.62	(-0.23, 3.48)	-0.07	(-0.80, 0.66)
p-trend	0.93		0.88		0.61		0.17		0.18		0.10	
DPHP												
Q1 (0.07-0.27)	-		-		-		-		-		-	
Q2 (0.28-0.65)	0.01	(-0.34, 0.37)	-0.29	(-0.60, 0.01)	-0.26	(-0.56, 0.05)	-0.19	(-0.50, 0.11)	-0.69	(-1.81, 1.03)	0.56	(0.04, 1.08)
Q3 (0.66-1.21)	0.94	(-0.83, 2.71)	-0.06	(-1.60, 1.48)	-0.23	(-1.75, 1.30)	-0.49	(-2.01, 1.02)	-0.47	(-7.64, 6.70)	1.26	(-1.41, 3.93)
Q4 (1.22-10.57)	-0.04	(-0.54, 0.47)	0.19	(-0.26, 0.63)	0.17	(-0.26, 0.61)	0.11	(-0.33, 0.55)	0.14	(-1.85, 2.12)	-0.60	(-1.35, 0.16)
p-trend	0.72		0.75		0.71		0.77		0.98		0.29	
ip-PPP												
Q1 (0.04-0.08)	-		-		-		-		-		-	
Q2 (0.09-0.18)	-0.02	(-0.14, 0.10)	-0.02	(-0.13, 0.08)	0.05	(-0.05, 0.16)	0.03	(-0.07, 0.13)	0.30	(-0.19, 0.78)	0.06	(-0.12, 0.25)
Q3 (0.19-0.45)	-0.03	(-0.27, 0.20)	-0.13	(-0.34, 0.07)	-0.04	(-0.25, 0.16)	-0.05	(-0.25, 0.15)	-0.15	(-1.10, 0.80)	0.22	(-0.15, 0.58)
Q4 (0.46-4.56)	0.48	(-0.60, 1.57)	0.17	(-0.80, 1.14)	0.11	(-0.83, 1.06)	-0.34	(-1.30, 0.62)	-1.58	(-5.76, 2.59)	0.65	(-0.95, 2.26)
p-trend	0.42		0.97		0.91		0.37		0.26		0.38	
∑OPE												
Q1 (0.17-0.79)	-		-		-		-		-		-	
Q2 (0.80-1.86)	-0.11	(-0.39, 0.18)	0.39	(-0.66, 1.44)	0.43	(-0.62, 1.48)	0.16	(-0.87, 1.19)	1.31	(-3.61, 6.22)	-1.16	(-2.93, 0.60)

Q3 (1.87-3.21)	0.02	(-0.31, 0.36)	0.34	(0.02, 0.66)	0.33	(0.003, 0.65)	0.32	(1.626x 10^{-6}, 0.64)	-0.19	(-1.63, 1.25)	-0.60	(-1.14, -0.06)
Q4 (3.22-15.56)	0.04	(-0.29, 0.37)	0.07	(-0.11, 0.24)	0.15	(-0.03, 0.33)	0.16	(-0.01, 0.34)	0.50	(-0.30, 1.31)	-0.12	(-0.42, 0.19)
p-trend	0.62		0.56		0.11		0.05		0.25		0.52	

[a] natural log transformation; Quartile 1=reference. Models adjusted for specific gravity, age, BMI, smoking status & abstinence period

Chapter V Appendix

Table V.A.1. Crude bivariate associations with OPE and semen parameters among men (n=220) from the EARTH cohort.

	OPE Metabolites											
	BDCIPP			DPHP			ip-PPP			tb-DPHP		
Semen parameters	r	25th, 75th %	p-Value	r	25th, 75th %	p-Value	r	25th, 75th %	p-Value	r	25th, 75th %	p-Value
Total sperm count	0.06		0.36	0.08		0.34	0.07		0.37	0.08		0.67
Concentration (mil/mL)	0.10		0.14	0.06		0.36	0.07		0.83	0.11		0.56
Motility (P+NP) (%)	**0.16**		0.02	0.08		0.23	0.04		0.61	0.11		0.55
Progressive motility	**0.15**		0.04	0.04		0.61	0.06		0.46	0.13		0.51
Morphology (%norm)	0.09		0.21	-0.02		0.82	0.05		0.55	0.29		0.12
Sample volume (mL)	-0.05		0.45	-0.05		0.43	0.004		0.96	0.14		0.45
Total sperm count <39 mill [a]	0.50	0.35, 0.76	0.13	0.72	0.34, 1.86	0.81	0.35	0.12, 0.83	0.55	0.13	0.13, 0.13	0.51
Yes	0.62			0.76			0.36			0.12		
No		0.35, 1.46			0.41, 1.47			0.21, 0.62			0.12, 0.22	
Sperm concentration <15 mil/mL [a]	0.46	0.35, 0.79	0.21	0.67	0.29, 0.93	0.21	0.30	0.13, 0.64	0.26	0.38	0.38, 0.38	0.22
Yes	0.62			0.77			0.37			0.16		
No		0.35, 1.16			0.42, 1.48			0.21, 0.64			0.12, 0.20	
Percent motile sperm (P+NP) <40 [a]	0.55	1.11	0.08	0.73	0.37,	0.68	0.36	0.21,	0.69	0.16	0.12,	0.80
Yes	0.72	1.67		0.74	1.37		0.39	0.59		0.16	0.20	

					0.42,			0.19,			0.12,	
No					1.47			0.64			0.25	
Percent morph.												
Sperm <4 [a]	0.62	0.36,	0.92	0.77	0.44,	0.82	0.36	0.20,	0.95	0.15	0.13,	0.21
Yes	0.61	1.26		0.74	1.32		0.40	0.79		0.18	0.16	
		0.34,			0.41,			0.21,			0.13,	
No		1.45			1.47			0.64			0.24	

r= spearman coefficient; [a] r = median values, p-value for 2-tailed test

Table V.A.2. Bivariate associations of OPE metabolite and demographic characteristics among men from the EARTH cohort (n=220).

Demographic characteristic	OPE Metabolites											
	BDCIPP			DPHP			ip-PPP			tb-DPHP		
	r	25th, 75th %	P-value	r	25th, 75th %	P-value	r	25th, 75th %	P-value	r	25th, 75th %	P-value
Age	-0.04		0.54	-0.01		0.81	0.03		0.69	-0.15		0.38
BMI	0.21		**0.0005**	0.08		0.15	0.04		0.49	-0.09		0.59
Abstinence period	0.07		0.27	0.01		0.89	0.08		0.26	0.14		0.45
Race [a]												
White	0.60	0.30,	0.08	0.74	0.41,	0.20	0.35	0.20,	0.38	0.16	0.09,	0.71
Black	0.99	1.29		0.53	1.28		0.97	0.61		0.23	0.20	
Asian	0.52	0.76,		1.23	0.25,		0.24	0.08,		0.16	0.23,	
Other	1.65	1.06		1.02	0.65		0.30	1.51		0.18	0.23	
		0.38,			0.55,			0.13,			0.09,	
		2.50			1.69			0.40			0.26	
		0.81,			0.73,			0.15,			0.18,	
		2.34			1.48			0.62			0.18	
Smoking Status [a]	0.62	0.30,	0.71	0.74	0.39,	0.67	0.34	0.19,	0.91	0.16	0.10,	0.75
Never	0.55	1.46		0.76	1.28		0.36	0.62		0.18	0.20	
smoke	0.58	0.37,		0.79	0.53,		0.35	0.20,		-	0.07,	
Past smoker		1.41			1.50			0.64			0.23	
Current		0.30,			0.46,			0.23,				
smoker		0.83			1.26			0.52				
Education [a]												
<High	2.66	0.56,	0.15	0.58	0.58,	0.51	0.36	0.24,	0.61	-	-	1.00
school	0.93	3.30		1.50	1.02		0.45	0.48		-	-	
HS grad	0.48	0.22,		1.30	0.48,		0.38	0.35,		0.16	0.16,	
1 or 2 yr.	0.74	1.52		0.68	4.32		0.47	0.62		-	0.16	
college	0.60	0.26,		0.74	0.54,		0.30	0.31,		0.16	-	
3 or 4 yr.	0.48	0.81		0.73	2.19		0.33	0.47		0.19	0.13,	
college		0.43,			0.47,			0.19,			0.17	
College grad		1.45			1.08			0.63			0.07,	
		0.38,			0.41,			0.16,			0.26	

Graduate degree		1.15 0.27, 0.13			1.24 0.34, 1.22			0.52 0.18, 0.54				
Season [a]			**<0.0001**			0.05			0.80			0.96
Winter	0.54	0.22, 0.98		0.89	0.54, 1.94		0.38	0.17, 0.54		0.15	0.10, 0.23	
Spring	0.44			0.71			0.34			0.17		
Summer	1.04	0.29, 1.81		0.75	0.34, 1.54		0.34	0.19, 0.77		0.16	0.10, 0.20	
Fall	0.60	0.75 0.52, 1.12		0.72	1.26 0.45, 1.03		0.35	0.63 0.21, 0.54		0.17	0.23 0.09, 0.22	

r= Spearman coefficient; [a] r = median values; Winter: December-February, Spring: March-May; Summer: June-August; Fall: September-November

Table V.A 3. Regression coefficients and odds ratios (95% CI) for semen parameters of men contributing (1-5) urine samples restricting 1.01 ≤ SG ≤ 1.03.

	OPE Metabolites [a]											
	BDCIPP			DPHP			ip-PPP			∑ OPE		
Sperm Parameter	B	95%CI	p-Value	B	95%CI	p-Value	B	95%CI	p-Value	B	95%CI	p-Value
Total sperm count [a, b]	-0.04	(-0.14, 0.07)	0.49	0.05	(-0.07, 0.17)	0.42	-0.0001	(-0.11, 0.00)	1.00	0.07	(-0.07, 0.21)	0.33
Concentration (mil/mL) [a, c]	-0.001	(-0.11, 0.10)	0.91	0.06	(-0.05, 0.17)	0.28	0.04	(-0.07, 0.14)	0.49	0.09	(-0.04, 0.23)	0.17
Motility (P+NP) (%) [a, c]	0.07	(-0.02, 0.17)	0.11	0.07	(-0.04, 0.16)	0.22	0.04	(-0.06, 0.14)	0.40	0.14	(-0.02, 0.29)	0.08
Progressive motility [a, d]	0.07	(-0.04, 0.17)	0.21	0.06	(-0.06, 0.17)	0.32	0.06	(-0.05, 0.17)	0.29	0.11	(-0.03, 0.25)	0.12
Morphology (%norm) [e]	0.32	(-0.07, 0.72)	0.11	0.22	(-0.26, 0.70)	0.36	0.26	(-0.19, 0.711)	0.25	0.41	(-0.17, 0.99)	0.16
Sample volume (mL) [f]	-0.10	(-0.26, 0.06)	0.20	-0.08	(-0.26, 0.11)	0.42	-0.06	(-0.24, 0.11)	0.47	-0.16	(-0.39, 0.06)	0.16
Odds Ratio	OR			OR			OR			OR		
Total sperm count <39 mill [b]	0.85	(0.63, 0.85)	0.33	0.90	(0.59, 1.39)	0.65	1.01	(0.55, 1.84)	0.98	0.71	(0.45, 1.12)	0.39
Sperm concentration <15 mil/mL [c]	0.88	(0.64, 1.22)	0.44	0.91	(0.57, 1.48)	0.70	0.98	(0.53, 1.79)	0.94	0.74	(0.41, 1.34)	0.39
Percent motile sperm (P+NP) <40 [b]	0.94	(0.72, 1.22)	0.64	0.92	(0.67, 1.27)	0.62	0.92	(0.66,1.28)	0.64	0.76	(0.52, 1.12)	0.17
Percent morph. Sperm <4 [e]	0.89	(0.65, 1.20)	0.43	1.00	(0.72, 1.39)	0.99	0.92	(0.65, 1.31)	0.64	0.81	(0.51, 1.27)	0.34

[a] natural log transformation; [b] n=168; [c] n=188; [d] n=185; [e] n=204; [f] n=214. Adjusted for specific gravity, age, BMI, smoking status & abstinence period

Table V.A 4. Intraclass correlation coefficients (95% CI) for repeated semen parameters among 220 men from the EARTH cohort.

Parameter	Subjects	Observations	ICC	95% CI
Total sperm count (mill)	174	235	0.52	(0.36, 0.67)
Concentration (mil/mL)	176	237	0.58	(0.41, 0.74)
Motility (P+NP) (%)	176	237	0.79	(0.69, 0.86)
Progressive motility	173	234	0.71	(0.58, 0.81)
Morphology (%norm)	189	255	0.51	(0.35, 0.66)
Sample volume (mL)	195	267	0.58	(0.43, 0.71)

Table V.A.5. Odds ratios (95% CIs) by quartile of OPE metabolite for males from the EARTH cohort contributing 1-5 samples.

		Semen parameters							
OPE [a]	(quartile range)	Total sperm count (<39 x 10^6)		Sperm Concentration (<15 million/mL)		Sperm motility (<40% motile sperm)		Morphology (<4% normal)	
BDCIPP									
Q1	(0.2-0.17)	-		-		-		-	
Q2	(0.18-0.51)	1.99	(0.65 6.14)	1.04	(0.38, 2.86)	0.69	(0.34, 1.36)	1.74	(0.79, 3.82)
Q3	(0.52-1.11)	1.24	(0.03, 59.36)	2.79	(0.05, 144.68)	0.21	(0.02, 2.96)	1.38	(0.08, 23.06)
Q4	(1.12-10.30)	0.16	(0.02, 1.42)	0.35	(0.05, 2.58)	0.99	(0.27, 3.58)	0.41	(0.08, 2.03)
p-trend		0.14		0.25		0.91		0.38	
DPHP									
Q1	(0.07-0.27)	-		-		-		-	
Q2	(0.28-0.65)	1.50	(0.32, 1.07)	1.15	(0.30, 4.42)	1.48	(0.57, 3.87)	0.63	(0.20, 1.97)
Q3	(0.66-1.21)	0.55	(7.90x 10^{-5}, 3847.58)	0.27	(1.03, 700.36)	0.13	(9.42x 10^{-4}, 18.52)	6.79 $x10^{-4}$	(1.72x 10^{-6}, 0.27)
Q4	(1.22-10.57)	1.04	(0.20, 5.31)	0.71	(0.07, 6.97)	0.76	(0.20, 2.95)	2.33	(0.40, 13.73)
p-trend		0.83		0.91		0.96		0.24	
ip-PPP									
Q1	(0.04-0.08)	-		-		-		-	
Q2	(0.09-0.18)	0.78	(0.43, 1.40)	1.02	(0.64, 1.62)	1.07	(0.76, 1.49)	0.89	(0.60, 1.34)
Q3	(0.19-0.45)	3.19	(0.71, 14.36)	2.70	(0.76, 9.53)	0.93	(0.48, 1.81)	1.07	(0.49, 2.36)

Q4	(0.46-4.56)	0.15	(8.92x 10^4, 26.62)	1.33	(0.01, 150.55)	1.53	(0.08, 31.26)	2.02	(0.08, 52.44)
p-trend		0.97		0.60		0.93		0.50	
∑OPE									
Q1	(0.17-0.79)	-		-		-		-	
Q2	(0.80-1.86)	1.60	(0.01, 502.90)	1.87	(0.01, 298.90)	1.02	(0.04, 29.15)	2.06	(0.04, 104.27)
Q3	(1.87-3.21)	1.57	(0.32, 7.71)	1.74	(0.42, 7.26)	0.71	(0.27, 1.85)	1.48	(0.45, 4.83)
Q4	(3.22-15.56)	0.79	(0.33, 1.86)	0.54	(0.20, 1.50)	0.76	(0.45, 1.29)	0.79	(0.37, 1.71)
p-trend		0.70		0.32		0.24		0.50	

[a] natural log transformation; Quartile 1=reference; Adjusted for specific gravity, age, BMI, smoking status & abstinence period

Chapter VI
Discussion

Summary of research findings

This dissertation highlights the lasting persistence of PBDEs and emerging presence of exposure to OPEs among a susceptible population. Using a novel study design, we were able to evaluate preconception FR exposure of females and their partners with intermediate and clinical IVF outcomes. Furthermore, our results also highlight the possibility of OPE use in consumer products. A summary of the main research findings for each aim is presented in Figure VI.1.

In the first aim, we evaluated the associations of five PBDEs and four OH-BDEs with IVF endpoints among a subset of 215 women from the EARTH study. PBDEs and OH-BDEs were frequently detected despite being phased-out of production. However, concentrations were highest for women recruited prior to 2010 compared to those who joined the study in 2010 or later. We did not observe any trends with PBDEs and OH-BDEs with intermediate IVF outcomes, although we identified some unexpected positive relationships of BDE153, 3 and 5-OH-BDE47, and 6-OH-BDE47 with implantation, clinical pregnancy, and live birth. Concentrations of PBDEs and OH-BDEs were higher in Other race women compared to White women in our stratified analysis. Despite

being underpowered, this provided insight to a possible racial disparity of PBDEs and reproductive health amongst Other race populations. Trends for Other race women were negative while associations among White women remained positive. BDE47 was associated with a decrease in the probability of implantation among Other race women.

The second aim expanded the first analysis to also include male PBDE and OH-BDE preconception concentrations among 189 couples. Detection frequencies were high for several PBDEs and OH-BDEs and similar to their female partners', concentrations for PBDEs and OH-BDEs were highest among men recruited in the early years of the study compared to those who joined in subsequent years. However, concentrations were higher in females compared to their partners. Correlations for congeners 47, 99, and 100 were strongest among couples, yet weakest for BDE153. Overall, no significant associations were observed for couple PBDE and OH-BDE exposure with IVF endpoints, yet some specific quartiles were associated with decreased probabilities of successful implantation, clinical pregnancy, and live birth.

The last aim focused on five OPE metabolites and was two fold. We first investigated the association of reported consumer product use with OPE metabolite concentrations in urine among a subset of 230 women and 229 men from the EARTH cohort. OPE metabolite concentrations were higher in women compared to men, yet metabolites BDCIPP, DPHP, and ip-PPP were frequently detected among couples. Correlations for metabolites among couples were weak. Reported used of moisturizer, nail polish, and nail polish remover among women was associated with elevated DPHP concentrations while conditioner, cosmetics, deodorant, and hair product use were

associated with an increase in BDCIPP concentrations. Mouthwash and vinyl glove use was associated with elevated DPHP concentrations in men.

In the final investigation, we evaluated the associations of OPE metabolites and semen parameters among a subset of 220 men from the EARTH cohort. OPE concentrations among each participant varied over time. Concentrations of BDCIPP samples varied by season and were 2-fold higher in the summer months compared to samples collected during other seasons. We observed a positive association with BDCIPP concentrations and sperm count. Although we identified some negative associations with sample volume and morphology in specific quartiles, overall associations were weak and inconsistent.

Integration of research findings

This dissertation uses a decade of data from couples seeking fertility treatment to provide insight to the persistence and possible associations of FRs with human reproduction. While concentrations of PBDEs and OH-BDEs declined over time, we observed a slight increase in OPE metabolite concentrations. Although overall associations with FRs were not significant, several overarching themes emerged from this dissertation:

1) **Concentrations of PBDEs decreased over time while OPE metabolite concentrations remained relatively stable.** A strength of this dissertation work is the benefit of monitoring the implications of a chemical phase-out during the study period. In the mid-2000s PentaBDEs were voluntarily removed from

products [1]. Although PBDEs bioaccumulate in our bodies and the environment, concentrations decreased over time for both women and their partners. However, among women we observed a steady decrease over the ten-year period with a noticeable drop in 2010 until the end of recruitment. We did not expect to observe a different trend among the male partners. Although concentrations decreased, the largest drop in concentrations occurred between 2006-2007 while concentrations remained stable for the remainder of the study period. While the phase-out is likely a result of declining concentrations among couples, it was unexpected to observe such a divergence in temporal trends of concentrations. As PBDE concentrations declined, we expected concentrations of OPE metabolites to increase, a trend which had been observed in a prior study of adults and children in the US [2]. However, metabolite concentrations for women and their partners remained relatively stable over the study period with a slight increase in BDCIPP in 2013 (Figure IV.2). Although we established some associations with OPE metabolites and consumer product use, it is possible that participants had not replaced old furniture, carpeting, etc. or they may have replaced these items with products that were FR free.

2) **Higher FR concentrations in women compared to their male partners.** Interestingly, we observed statistically significant higher concentrations of all FRs and metabolites for women compared to their male partners. Similarly, correlations for each FR and metabolite was also weak among couples. Weak correlations and differences in OPE metabolite concentrations is possibly due to

the rapid metabolism of parent compounds once in the body, or as we observed in the third aim, OPE exposure could be attributed to consumer product use which can be both episodic and vary by gender [4,5]. However, considering the common sources of exposure for PBDEs among couples (i.e. indoor environments), the differences in exposure were unexpected [6]. However, some studies have observed varying PBDE concentrations in different rooms of a house [7]. It is also possible the differences in concentrations arise from separate workday environments, as it is unlikely that couples also work in the same location together. Differences in BDE153 have been associated with specific diets which could possibly account for the difference in concentrations and the weak correlations, as diets vary by individual [8]. Interestingly, unadjusted PBDE and OH-BDE concentrations were comparable among women and men and we also observed statistically higher BMI and lipid levels in men compared to women. Therefore, lipid concentrations may be vital in PBDE and OH-BDE exposure. Lipid peroxidation may be increased in women during IVF which may activate a release of chemicals within fat stores [9].

3) **Unexpected positive associations of female PBDE and OH-BDE concentrations with clinical IVF outcomes.** The hypothesis from the first aim was established from several existing laboratory studies that had observed greater toxicity among OH-BDEs compared to PBDEs, yet our results were contrary to those findings. Not only were associations positive for PBDEs with clinical outcomes, associations with OH-BDEs were positive and at a greater

magnitude than PBDEs. The positive results were unexpected yet do not remain when accounting for male exposures. Unmeasured confounding regarding diet is possible as some OH-BDEs are organically produced in marine environments [10]. Finally, the continued increase in IVF success rates over time could have also biased our results in both female and couple models by overestimating the positive relationship between successful implantation, clinical pregnancy, and live birth rates with PBDE and OH-BDE concentrations [14].

4) **The inclusion of male exposure when evaluating the relationships of environmental toxicants and infertility is essential.** While there are many existing cohorts assessing the impact of environmental toxicants and reproductive outcomes among women [15–17], few reproductive health studies focus on either male or couple exposure, yet primarily focus on TTP [18–21]. The EARTH study design enables the assessment the associations of preconception exposure of environmental toxicants with reproductive endpoints from preconception to live birth. Merely accounting for female exposure could possibly over or underestimate the impact of a chemical exposure on fertility and pregnancy outcomes. In the second aim, when also accounting for male exposure, the positive results with implantation, clinical pregnancy, and live birth diminished and lost statistical significance. The addition of male exposure also increases the ability to evaluate a larger scope of the possible mechanisms of environmental toxicants on reproduction using epidemiologic data.

Impact and innovation

Human reproduction is a composite of multiple biological responses, many of which typically occur without observation. Epidemiologic studies assessing infertility solely through fecundity and TPP in the general population fail to distinguish the many endpoints between pre-conception and live birth. Using a population undergoing IVF allows for the capture of intermediate as well as clinical birth outcomes. Isolating each endpoint allows us to differentiate critical windows that may inhibit fertility. Also, this dissertation incorporates, the contribution of PBDEs and OH-BDEs exposures as a 'couple,' to better elucidate the role of exposure from both sexes during reproduction. Assessing the relationship between male and female exposure also provides insight to possible routes or exposure pathways and potential diverging mechanistic pathways. Correlations among all FRs and metabolites were weak among couples and suggests distinct sources of exposure per individual, especially in respect to those living in the same household. Using data spanning nearly a decade, this dissertation work was able to assess the temporal trends of a class of chemicals undergoing a phase-out as well as the addition of an alternative compound. Our use of CWGEE to model the relationship of PBDEs and OH-BDES with clinical outcomes is a novel statistical approach to provide more precise effect estimates while accounting for multiple cycles per women or couple [22]. Finally, studies assessing the relationship between OPEs and consumer products are limited [4,23]. Our comprehensive analysis assessed the relationship of PCP and HP with OPE metabolites. Our findings of positive associations between OPE metabolites and use of several PCPs among women facilitates future, more specific studies targeting OPE concentrations in consumer products.

Recommendations for future research

This dissertation has several strengths including novel study design, innovative statistical methodologies, and targeted assessment of a susceptible population which contributes to the current scientific body of evidence of FRs and human reproduction. However, our comprehensive results and study limitations provide opportunities for future research:

1) **Target more diverse IVF study populations.** Patients from fertility clinics often have limited ethnic and racial diversity. The high cost of IVF and few states with insurance mandates for fertility treatment typically limit studies to highly educated, primarily White participants. The demographics from our sample follow these assumptions with approximately 80% of patients identifying as White and most having a college or graduate degree. One study in the United Kingdom (UK) of over 13,000 first IVF cycles observed poorer IVF outcomes among Black women compared to White [24]. Higher PBDE and OH-BDE concentrations have been observed among Black women compared to White women in a cohort in CA [25]. Unfortunately, our sensitivity analysis stratifying by race was underpowered, yet associations with clinical outcomes became negative when stratified for Other race women. It is possible that a larger and more diverse IVF population could provide insight to a possible racial disparity among the relationship of PBDE and OH-BDEs with fertility.
2) **Reproductive toxicity of emerging FRs and FR mixtures.** This dissertation concentrates on two primary classes of FRs, yet there are several others

including halogenated FRs, novel brominated FRs (NBFRs), and dechlorane which have all been detected in indoor dust samples [26,27]. Human studies evaluating the association of these alternative FRs are limited, yet mixtures of bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) and 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) have been associated with reduced fecundity among Japanese medaka [28]. Despite the crucial necessity to understand the specific relationships between individual FRs and human reproduction, as multiple FRs are highly detected across the general population, a comprehensive understanding of the reproductive toxicity of FR mixtures is necessary. Furthermore, the understanding of how our complex daily microenvironments of mixtures including but not limited to: FRs, phthalates, metals, and particulate matter comprehensively impact reproductive health is also essential.

Final comments

This dissertation highlights the continued persistence of PBDEs and their metabolites, despite their being phased out of production. However, this longitudinal analysis was able to capture temporal trends using ten years of FR exposure sample collection to provide perspective of declines in PBDE concentrations and slight increases of OPE concentrations among couples seeking fertility treatment. Although our results were unexpected, few studies to date have investigated the association of OH-BDEs and reproductive health. Furthermore, our novel study design underscores the importance of considering male exposures when evaluating the relationships of environmental toxicants with fertility and pregnancy outcomes. The third aim of this

dissertation illustrates the cyclical relationship of harmful substitution chemicals and demonstrates their use in products other than as a FR. This study was also the most comprehensive analysis of consumer products and OPE metabolites. We observed several associations of metabolites with consumer product use among men and women. It is possible consumer product use also contributes to internal OPE exposure. Although FRs were initially developed to provide important safety benefits, this work contributes to the growing body of evidence of the persistence and possible reproductive health effects of FRs and calls for the development of safer alternatives or discontinuing the addition of FRs to consumer products.

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Figure VI.1. Conceptual diagram of specific aims, main research findings, and public health impact.

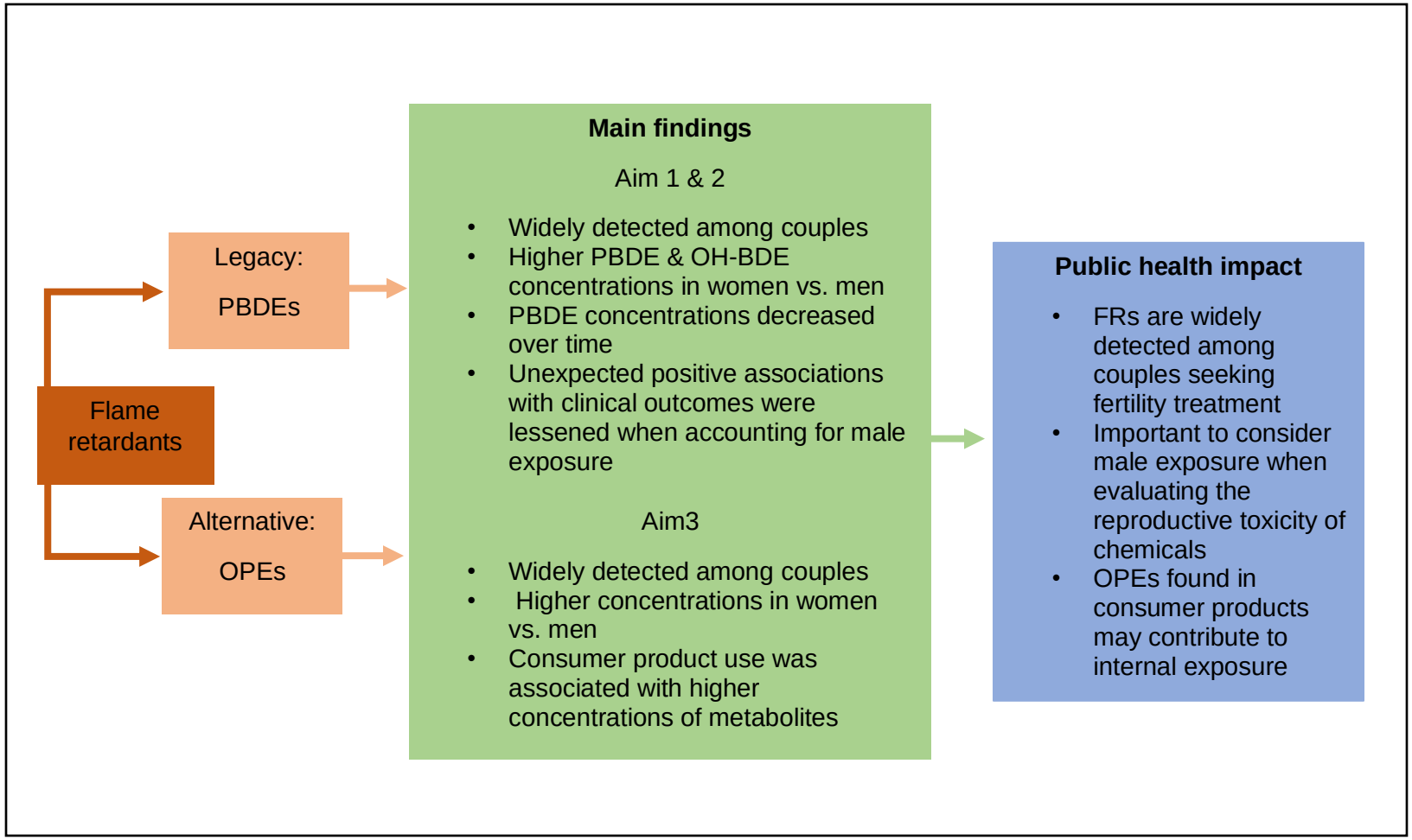

Figure IV.2. Geometric means of OPE metabolite concentrations (µg/L) stratified by year of sample collection among a subset of men (n=229, n=335 urine samples) and women (n=230, n=638 urine samples) from the EARTH cohort.